Spontaneous baroreflex sensitivity for risk stratification of heart failure patients: optimal cutoff and age effects*

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Abstract (max 300 words)

Baroreflex sensitivity (BRS) is an important prognostic factor as a reduced BRS has been associated with an adverse cardiovascular outcome. The threshold for "reduced" BRS was established by the ATRAMI study at BRS <3 ms/mmHg in patients with a previous myocardial infarction and has shown to improve risk assessment in many other cardiac dysfunctions. The successful application of this cutoff to other populations suggests that it may reflect an inherent property of baroreflex functioning. Hence, our goal is to investigate whether it represents a "natural" partition of BRS values. Since reduced baroreflex responsiveness is also associated with aging, we also investigate whether a BRS estimate below 3 ms/mmHg can be the result of a process of physiologic senescence besides a sign of BRS dysfunction.

This study involved 228 chronic heart failure (CHF) patients and 60 age-matched controls. Our novel method combines transfer function BRS estimation and automatic clustering of BRS probability distributions to define indicative levels of different BRS activities. The analysis produced a fit clustering (cophenetic coefficient 0.9 out of 1) and identified one group of homogeneous patients (well separated from the remaining by 3 ms/mmHg) with increased BRS based mortality risk (HR: 3.19 [1.73,5.89], p<0.001). The age dependent BRS cutoff, estimated by 5% quantile regression of log(BRS) with age (considering the age-matched controls), provides a similar mortality value (HR: 2.44 [1.37,4.43], p=0.003).

In conclusion, the 3 ms/mmHg cutoff identifies two large clusters of homogeneous HF patients, thus supporting the hypothesis of being a natural cutoff in the HF population. Furthermore, age was found to have no statistical impact on risk assessment, thus suggesting that there is no need to establish age-based cutoffs as 3 ms/mmHg optimally identifies patients at high mortality risk.

Summary statement

This study provides statistical support for 3 ms/mmHg as a cutoff value that best identifies a homogeneous group of patients with depressed BRS and highlights the robustness of this threshold in risk stratification due of its age-independent prognostic value.

Abbreviations: BRS, baroreflex sensitivity; CHF, chronic heart failure; HR, hazard ratio; HF, heart failure; SBP, systolic blood pressure; LVEF, left ventricular ejection fraction; MI, myocardial infarction; TF, transfer function; pdf, probability density function; CI, confidence interval.

Clinical perspectives

Previous studies have shown that a baroreflex sensitivity (BRS) <3 ms/mmHg identifies cardiac patients at higher mortality risk. This study aims at assessing whether this threshold represents a natural partition of the data and whether its risk stratification capability is dependent of age.

- We found in a large cohort of heart failure patients that 3 ms/mmHg best identifies two groups with homogeneous BRS and those with a BRS <3 ms/mmHg show increased cardiac mortality risk, independently of age.
- Novel interventional and device-based therapies aimed at modulating the autonomic nervous system are under investigation in cardiac conditions associated with sympathetic overactivity and autonomic imbalance. The individual characterization of the arterial baroreflex regulation and the recognition of a clinically meaningful dysregulation of the arterial baroreflex control offer a number of opportunities in identifying patients who may better benefit from treatment and in tracking the effects of the applied therapies over time.

Introduction

It is well-established that the joint analysis of beat-to-beat fluctuations in systolic blood pressure (SBP) and RR interval series provides a useful indicator of the arterial-cardiac baroreceptor reflex sensitivity (BRS), expressed in milliseconds per millimeters of mercury units (ms/mmHg) [1]. The BRS has been extensively studied in cardiac patients [2], particularly in those surviving an acute myocardial infarction (MI) but also in those with heart failure (HF) or with left ventricular (LV) dysfunction. The majority of studies have shown that lower BRS values are associated with higher cardiovascular disease-related mortality within a few years after the event or the diagnosis [3, 4, 5]. However, there is no objective criterion to distinguish between different levels of BRS function and, therefore the definition of depressed BRS has been commonly referred from the Autonomic Tone and Reflexes After Myocardial Infarction (ATRAMI) study [3], which was the first study to provide cutoff values for risk stratification according to BRS values. The ATRAMI was a large scale prospective study including 1284 subjects with a recent MI and aimed at the identification of significant risk factors for cardiac mortality, including the phenylephrine BRS and the traditional parameter left ventricular ejection fraction (LVEF). The results pointed out that reduced BRS values increase risk of cardiac related mortality independently of LVEF, with risk classes defined according to the sample BRS distribution: high risk below the 15-th percentile (BRS<3.0 ms/mmHg), medium risk between the 15-th and the 50-th percentile ($3.0 \leq$ BRS ≤ 6.1 ms/mmHg) and low risk above the 50-th percentile (BRS > 6.1 ms/mmHg). The 3.0 ms/mmHg cutoff, was also shown to be suitable to predict 5-year mortality in MI-patients with preserved LVEF or age above 65 years [3].

Even though the ATRAMI cutoffs were determined in post-MI patients, such cutoffs (in particular, 3.0 ms/mmHg) have been used also for non-ischemic dilated cardiomyopathy patients [6] and HF patients including those under beta-blockers treatment [7], with BRS assessed by the phenylephrine technique. Surprisingly, when BRS is estimated through bivariate spectral analysis of spontaneous oscillations of systolic arterial pressure and RR intervals [8], the cut-off value for BRS maximizing the hazard ratio for 2-years outcome was found to be 3.1 ms/mmHg. This value, obtained for transfer function BRS assessment, is remarkably close to the one obtained for the phenylephrine test. Indeed, transfer function and phenylephrine methods provide estimates with poor agreement although with similar predictive value considering the 3.0 ms/mmHg cutoff in HF subjects [9].

Recently, Huikuri and colleagues [10] pointed out the need to define an algorithm for determining the ideal cutoffs for different measures of heart rate variability. Concerning the

BRS, the finding that the 3.0 ms/mmHg cutoff has been successfully applied in most studies and with different methodologies, suggests that this threshold might actually have a physiological meaning. The need for determining "ideal" cut-off values of autonomic variables is particularly relevant in the setting of HF patients in whom a device-based approach to autonomic nervous system modulation is under investigation for the treatment of the disease. Although clinical studies obviously rely on clinical outcomes, to define whether these new interventions are biologically active on their physiological end-point (i.e. the autonomic balance) might help in identifying those patients who might better benefit of further attention.

The first goal of the present study is to investigate whether the 3.0 ms/mmHg cuttoff is representative of a natural partition of the data into clusters of homogeneous subjects. The study is based on automatic agglomerative clustering of individual BRS estimated probability density functions, thus, considering the BRS estimate as well as the corresponding intra-individual BRS variability. Therefore, this methodology is independent of the patient outcome and solely based on the most likely BRS values for each subject.

The second goal is related to age effects. It is known that BRS decreases with age [11, 12, 13], and age specific reference BRS values have been reported for a healthy working population (<60 years) and for older subjects (>50 years) [11, 12]. Thus, we investigated the possibility that the optimal cutoffs may vary as a function of the age of the subject, a possibility that so far has not been considered in the literature.

Experimental data

Study patients. This is a retrospective analysis conducted on recordings from a group of 228 HF patients collected within a previous study [8]. Shortly, 84% of the patients were male and the median age of the group was 54 years (ranging from 26 to 70 years). The subjects distribution per NYHA functional class (I, II, III, IV) was 10%, 51%, 37% and 2%, respectively, and per cause of CHF (ischemic, idiopathic, other) was 48%, 40% and 12%. The patients were all clinically stable, had not had a myocardial infarction or cardiac surgery within the last six months, were in sinus rhythm and had a low ectopic event rate (< 5%). We also considered a group of 60 healthy subjects in the same age range (from 26 to 66 years), selected from those enrolled in previous investigations from the same laboratory. None of them were on medication or suffered from chronic or acute disease.

Experimental protocol. Subjects were studied in the morning in the supine position. The experimental protocol was carried out in the laboratory for autonomic evaluations in Montescano and comprised: 1) instrumentation, patient's familiarization with paced breathing and signal stabilization; 2) 8-min recording of an electrocardiogram (ECG), lung volume (Respitrace Plus, Non-Invasive Monitoring Systems, Miami Beach, FL, USA), and non-invasive arterial blood pressure (Finapres 2300, Ohmeda, Louisville, CO, USA) at a 250 Hz sampling frequency and during paced breathing at 15 breaths/min (0.25 Hz). Beat-by-beat RR interval values (resolution 1 ms) were obtained from the ECG signals using an in-house developed software package [14]. The RR interval time series were then resampled at 2 Hz by cubic spline interpolation. The local Ethical Committee had approved the study. All patients provided written consent for the scientific treatment of their data in an anonymous form at the time of hospitalization. Normal subjects also provided an informed written consent at the time of enrollment.

Patients followed recorded instructions to breath in and out at a frequency of 0.25 Hz, with the inspiratory duty cycle (inspiratory time (TI) / total breath time (TTOT)) set at 0.4 [15]. A short familiarization session was carried out before beginning the recording session. The reason to adopt the 0.25 Hz paced breathing protocol for BRS evaluation was twofold. First, the voluntary control of respiration at 0.25 Hz avoids the quantification of respiratory effects (mechanically on systolic arterial pressure and centrally on heart rate) in the LF band [15]. In heart failure condition, about 50% of patients develop a periodical breathing pattern during supine laboratory recordings [16], which causes a profound entrainment of cardiovascular rhythms [17]. Thus, voluntary control of respiration at frequencies outside the LF range is used as a means to avoid this problem. Second, it is easier for a subject to follow a regular breathing pattern rather than to keep the breathing rate within given limits. Finally note that, despite a slight hyperventilation, BRS evaluated under 0.25 Hz paced breathing showed no significant differences to BRS measured during spontaneous HF breathing (0.15-0.40 Hz) both for normal subjects and heart failure patients patients [15, 18].

Methods

1. BRS estimate and probability distribution

BRS was evaluated by bivariate spectral analysis between SAP and RR time series and averaging the estimated gain of the transfer function (TF) in the 0.04-0.15 Hz range [19]. Spectral estimates were obtained via Blackman-Tukey algorithm considering a Parzen

window [20] with 0.03 Hz bandwidth [8, 21]. In addition to the point BRS estimate, we estimated the BRS probability density function (pdf) for each subject, in order to define an individualized range of likely BRS values. The pdf is based on approximate 100 (1 - a)% confidence intervals (CI) for the TF gain per frequency using the weighted covariance estimator [22], which have been found to be accurate enough to be used in practical BRS analysis [21]. For each frequency value, the 100 (1 - a)% CI of the TF gain was quantified by varying 1 - a from 0.01 to 1 (stepwise 0.01), where the case a=1 corresponds to the point TF estimate. Then, the pdf percentile limits 100 (1 - a)% were obtained by averaging the 100 (1 - a)% CI limits over all frequencies, such that the case a=1 corresponds to the BRS estimate for each subject. With this procedure, the pdf for the BRS of each subject was evaluated at 99 points uniformly spaced over the probability range [0,1[.

2. Clustering Methodology

The starting point of the clustering procedure is the panel of BRS pdf estimates for each subject. This procedure builds the hierarchy from the individual elements by progressively merging clusters, using an appropriate dissimilarity measure and a group linkage criterion [23]. In this work, the dissimilarity matrix D has entries D_{ij} that correspond to the pairwise dissimilarity between the subjects i and j, and quantified by the weighted L_2-Wasserstein distance. This distance corresponds to a weighted sum of squared differences between quantiles of distributions i and j and, therefore, the dissimilarity between two subjects takes into account the differences between the BRS estimated probability distributions and the differences between the mean/median BRS behavior of the subjects (i.e. the point BRS estimate).

A dendrogram was then obtained by applying the group linkage criterion to the matrix I-D. The dendrograms obtained by the different linkage procedures (single, complete and average) were compared by the cophenetic correlation coefficient which is a measure of how faithfully the linkage preserves the pairwise distances between the original data (see, e.g. [23] page 91). Values close to 1 indicate that the linkage/dendrogram accurately reflect the data.

3. BRS distribution and prognostic as a function of age

To determine the impact of using an age-dependent BRS cutoff in prognostic studies instead of a constant threshold, we addressed two aspects. Firstly, we investigated whether values of BRS below the ATRAMI cut-off value (3.0 ms/mmHg) can be considered either the result of

a process of physiologic senescence or a sign of BRS dysfunction. This was accomplished by comparing BRS as a function of age in CHF patients and in age matched control subjects. Secondly, we assessed the performance of age dependent cutoffs in the outcome prognostic of CHF patients, as compared to the constant 3.0 ms/mmHg value. The statistical analysis was based on logarithmic transformed BRS values due to the substantial positive skewness of BRS distribution per group (and lack of normality, as assessed by Kolmogorov-Smirnov testing) as well as to enhance linearity between BRS and age [24]. The age effect on BRS in the HF and in the control groups was compared by analysis of covariance (ANCOVA) with age acting as a covariate. Furthermore, the distribution of log BRS per age in the HF group was analyzed with respect to the control group as follows. Quantile regression was used to specify the 5% and the 95% conditional quantile functions of log(BRS) given age in control subjects [25] and to determine the interval in which 90% of the control subjects are included within. Then, the proportion of HF and of control subjects in each region were compared by independent samples t-test with Bonferroni correction on bilateral hypothesis testing. A comprehensive summary of the theoretical formulation and estimation of parameters in quantile regression can be found in Gouveia *et al*. [26].

The predictive value of BRS depending on age was firstly assessed by Cox analysis with age as a continuous adjusting factor and the estimation of Hazard Ratios (HR) and corresponding 95% confidence intervals. Unless stated otherwise, all results are presented as value [95% CI]. Kaplan-Meier analysis was used to estimate survival curves for the age specific threshold based on the 5% quantile line from the control group, R5%, and compared with the constant log(3.0) threshold. Survival curves from different subgroups were compared by log-rank test (Mantel-Cox) and multiple comparisons with Bonferroni correction. A p-value <0.05 was considered as statistically significant and all tests were two-sided.

Results

The dendrogram in Figure 1a shows the hierarchy from the individual elements by progressively merging clusters. It shows how subjects are linked; the lower the linkage level, the higher the similarity between subjects. The pairs of subjects exhibiting the lowest distances are represented at the first level nodes of the dendrogram. At higher level nodes of a dendrogram, the distance between clusters of distributions is higher, reflecting the larger variety of BRS distributions for the subjects included in that cluster. The grouping criteria considered to build the dendrogram in Figure 1a was the average linkage procedure since it

exhibits the highest cophenetic correlation coefficient (0.90). Since this value is quite close to 1, the produced clustering is a close fit, thus properly reproducing the original matrix distance D between subjects. The average linkage method also shows to be less dependent upon extreme values and producing clusters with small within cluster variation and with approximately equal variances.

The clustering procedure allows the identification of groups of homogeneous subjects. In particular, Figure 1a highlights in green color a group of subjects with BRS distributions showing high similarity within the group and low similarity with the BRS distributions of the remaining subjects. As displayed in Figure 1b, the colored group corresponds to the subjects with lowest mean BRS estimate and lowest variability in BRS distribution. Moreover, the empirical cutoff of 3.0 ms/mmHg well separates the subjects of the colored group from the remaining ones.

(Figure 1 about here)

BRS distribution is age dependent. The log BRS evaluated in the HF group significantly lower than that of the control group (mean±SD, 1.18±0.96 vs 1.91±0.66, p-value<0.0001, Figure 2a). Due to the fairly symmetric shape of the log-transformed distributions (Figure 2a), the exponential of the log BRS average (i.e., the geometric mean of the BRS values) well estimates the median of BRS values in ms/mmHg units (3.25 [2.86,3.68] vs 7.78 [5.73,8.04]). As illustrated in Figure 2b, log BRS correlated inversely with age in both groups, although the correlation is weaker in HF than in control group (table 1). However, the larger sample size in HF group makes possible a nearly equivalent estimation precision in both groups and, consequently, regression confidence bands per group are of similar width (Figure 2b). Statistical ANCOVA analysis provided no significant interaction between age and group (p-value=0.46) indicating that HF and control slopes are not significantly different and thus log BRS and age relate similarly in both groups. The joint regression analysis for HF and control groups indicate that the coefficients associated with age, intercept and group are significant in the regression model (table 1). Therefore, HF subjects were found to exhibit a lower log BRS value in comparison with controls, differing by a constant factor of 0.562 [0.44,0.75] after adjusting for age.

(Figure 2 about here)

The log BRS distribution per age was also compared among the different groups. Figure 3a displays the 5% and the 95% conditional quantile lines obtained for the control group (R5% and R95% with equations in table 1), dividing the controls in two sets (below/above the line) where below the line are approximately 5% and 95% of the subjects, respectively. Therefore, the region in between R5% and R95% contains approximately 90% of the controls. As illustrated in Figure 3b, the proportion of HF patients below R5% is larger than that observed for control subjects (76/228 vs 4/60, p-value<0.001, table 1) while there are no significant differences between those proportions evaluated above R95% line (6/228 vs 4/60, p-value=0.13, table 1). Thus, the lower log BRS average in HF when compared to controls is a consequence of the lower minimum and similar maximum log BRS values per age in HF when compared to controls. Finally, the proportion of HF subjects with BRS below 3.0 ms/mmHg is larger than in controls (98/228 vs 7/60, p-value<0.001, table 1) and larger than that evaluated for HF subjects below R5% (98/228 vs 76/228, p-value=0.017, table 1).

(Figure 3 about here)

BRS prognostic value is not age dependent. In the HF group, Cox regression analysis shows that log BRS is significantly associated with the outcome (HR: 0.61 [0.47,0.79], p-value<0.001), thus indicating that lower BRS values are associated with cardiac mortality. With age as the adjusting factor in a multivariate Cox model, log BRS maintains a highly significant association with the outcome (HR: 0.61 [0.47,0.80], p-value<0.001) while there was no evidence of age impact on the outcome (HR: 1.01 [0.98,1.05], p-value=0.48). The log BRS values dichotomized in two regions are also associated with outcome either considering the log(3.0) threshold (HR: 3.19 [1.73,5.89], p-value<0.001) or the age dependent threshold (HR: 2.44 [1.37,4.43], p-value=0.003); in both cases, patients with log BRS values below the threshold show increased cardiac mortality risk. Moreover, the mortality prognoses in HF subjects either based on the constant or the age dependent threshold were found to be similar (Figure 4). Notice that such a result is expected because the value of log(3.0) is intersecting the age-based threshold R5% roughly in the middle of the age range (around 45 years, see Figure 3) and the slope of log BRS over age regression line is not too steep. Therefore, the patients falling in the region below the thresholds (either log(3.0) or R5% defining the high risk region) are approximately the same (Figure 3b).

(Figure 4 about here)

Survival functions were also obtained when considering 4 distinct regions (see Figure 5a), in order to investigate mortality risk for the patients with different risk at log(3.0) and at R5% criteria. Patients at high risk for log(3.0) criterion and at low risk for R5% criterion are identified in yellow (region 3). Green identifies patients in region 2, i.e., a region with the same size as region 3 including patients of the same age (patients with log BRS in between log(3.0) and the line obtained by reflection of R5% at log(3.0), ie log BRS=-0.026*age-2.19+2*log(3.0)). Finally, 1 and 4 correspond to regions of low and high risk of mortality respectively, considering both criteria. As observed in Figure 5b, the survival in region 3 is similar to that of region 4 (p-value=0.90) while significantly different from that of the regions 2 and 1 (p-value=0.038 and 0.031, respectively). Finally, estimated survival rate is higher in region 2 than in region 1 although not exhibiting significant differences (p-value=0.55).

(Figure 5 about here)

Discussion

There are two main points to highlight. First, the results of this study suggest that the 3.0 cutoff on BRS estimates represent a natural partition of heart failure patients at risk. Second, the 3.0 cutoff is rather independent of age in patients with heart failure.

The results of our study are relevant in the current clinical scenario where a number of different approaches including baroreceptor activation therapy, have been developed to modulate those autonomic abnormalities that characterize heart failure. While it is assumed that all these procedures would act upon a deranged autonomic balance to improve it, a systematic evaluation of the effects of such therapeutic options on the autonomic profile of the treated patients has not been performed. The appropriate identification of patients who can benefit from these procedures is still an unsettled issue that requires evaluation.

Clinical value of the 3.0 cut-off

Patients with BRS estimate lower than 3.0 were shown to exhibit higher mortality risk in several literature studies [1, 3, 5, 6, 7]. Such threshold seems to be constant through different methodologies. In ATRAMI and also in other studies, baroreflex sensitivity has been assessed by the drug infusion technique with phenylephrine. The 3.0 cut-off value of the

phenylephrine method is close to the 3.1 cut-off value obtained by a very distinct method, in which the transfer function method was used for BRS evaluation [8].

The results in our study support the concept that a cut-off value around 3 ms/mmHg can be viewed as, say, a *biological threshold* for the functioning of the baroreflex. Actually, in this sample of HF patients, the 3.0 ms/mmHg cut-off value represents the intrinsic grouping of BRS distributions. This is quite clear from the visualization of the results in the dendrogram and corresponding estimated pdf distributions (Figure 1), where it is possible to observe that the group with highly similar pdfs (Figure 1a) constitutes the group of patients exhibiting a punctual BRS estimate lower than 3.0 ms/mmHg (Figure 1b).

The natural partition of BRS data around 3 ms/mmHg also suggests that below this level a proper functioning of the baroreceptor reflex is no longer present and the RR interval changes (if any) are no longer linearly related to blood pressure oscillations. This is also exampled by the observation that a coherence value > 0.5, generally taken to satisfy the assumption of linearity, is often not found for the lower estimates of BRS.

Studies with ganglionic blockade also support the concept that in the presence of functional denervation the transfer function gain is substantially decreased but not completely abolished. Zhang at al [27] calculated the mean value of the transfer function gain in the LF band in resting humans after complete ganglionic blockade. This is equivalent to measuring BRS according to the method used in our study after functionally opening the baroreflex arc. They found that BRS was 0.9±0.1 ms/mmHg (mean±SEM) in a sample of 10 subjects. Assuming that ganglionic blockade was fully effective in all subjects, 1.52 ms/mmHg represents an estimate of an upper bound of biological/methodological noise considering a 95% range of variation in the BRS values of the sample. Thus, a BRS value < 3.0 ms/mmHg would be indicative of a complete impairment or of a marked attenuation of the baroreceptor-heart rate reflex.

BRS and age

It is largely acknowledged that increasing age significantly reduces the baroreflex control of heart rate. The decline of BRS with age was first described by Gribbin et al. [28] and was subsequently confirmed by several studies that consistently showed that the relation with age is diminished or lost beyond the age of 60 suggesting that the majority of the reduction seen in BRS with age has already occurred by the fifth decade [29, 30, 31, 32]. Recent studies showed that decreasing baroreflex sensitivity with aging is not due to efferent autonomic dysfunction [33].

As expected in our study, log BRS is significantly lower for HF than for control group, being inversely correlated with age in both groups. To our knowledge no literature is available aiming at exploring the linear association between BRS estimates and age for HF patients, although it is expected that the BRS would decrease with age. In the present study, there was no significant interaction between log BRS and group thus indicating that BRS decay/slope with age is similar in HF and controls and, consequently differences between groups are constant across all ages. Different results were obtained in a recent study when male patients with recent MI were compared with age-matched controls [5]. Although the BRS slope was found to be significantly lower in MI patients, the decrease of BRS associated with age was significantly steeper in controls than in MI patients. Unfortunately, we do not have a tenable explanation for this disparity. It is possible, however, that the limited number of control subjects in the study of Hartikainen et al. and the inclusion of younger subjects (range 30 to 69 years) might have resulted in an overestimation of the BRS values in younger subjects (due to the increased inter and intraindividual variability at higher BRS value).

The results concerning the correlation between BRS and age in controls (n=60) are in accordance with other literature studies with groups of larger size (e.g., n=1026 in [11]), which support their use as a reference group in this study. Namely, Figure 3a represents the BRS estimates for the normal subjects as a function of age and evidences the tendency that BRS decreases significantly with increasing age (table 1). In particular, Kardos et al. [11] obtained a log BRS age-related regression line with similar parameters (log BRS = -0.027*age+3.24, n=1026, r2=0.23, p-value<0.0001) in a much larger group of healthy working subjects between 18 and 60 years old. The slightly higher value at the origin provided by Kardos et al. [11] in comparison with the present study (3.24 vs 3.13, with no significant differences), may be attributed to the differences in BRS estimation approaches and breathing protocol. Specifically, Kardos et al. [11] quantified the BRS through the sequences technique and spontaneous breathing, which are factors that were shown to provide higher BRS estimates than those obtained by spectral analysis and controlled breathing at 15/min [12, 15, 34, 35]. Moreover, the age dependent thresholds obtained in this study (R5% and R95%) are lower than those depicted from [11] showing similar variability of the log BRS values around the age dependent regression line (r2=0.28 in this study and r2=0.23 in [11]). These arguments also support the idea that the differences between the studies reflect the different approaches to estimating BRS.

BRS cut-off value and prognosis

Because reduced baroreflex responsiveness is also associated with aging, age was introduced as a covariate to investigate if cutoff values vary as a function of age. First, as assessed by Cox regression, log BRS was significantly associated with the outcome. When age was considered in the regression, log BRS maintained a significant association with outcome whereas age had no significant impact. Second, log BRS values dichotomized in two regions (either with the log(3.0) or the age based R5% criteria) had significant association with outcome, because both cutoffs identified fairly the same patients at high risk. To further investigate differences between cutoffs, the subjects at risk for R5% criteria and not for log(3.0) were considered separately (mainly the older subjects) and were found to exhibit a lower mortality risk, similar to that evaluated for the patients with log BRS higher than log(3.0) and similar age. Therefore, our results do not indicate the need to establish an age-based cutoff. Rather, they corroborate the hypothesis that log(3.0) is a natural threshold in HF population able to optimally identify patients at high mortality risk.

Conclusions

This study provides mathematical and statistical support for the *age-independent biological value* of 3 ms/mmHg, which has been used for defining a depressed BRS. Our analysis shows that this threshold value represents a natural partition in the HF population, through BRS probability functions (including punctual BRS estimates and intraindividual variability) obtained from the analysis of spontaneous oscillations in heart rate and blood pressure. Thus, these data reinforce the clinical applicability of this measure not only for risk stratification purposes but also as a means of tracking effects of therapeutic interventions targeted at improving autonomic modulation and baroreceptor activity.

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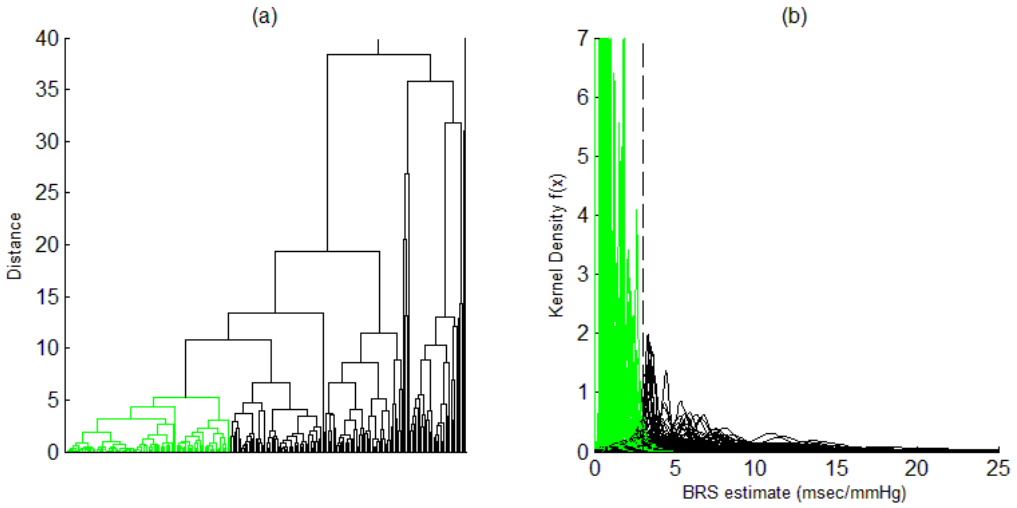

Figure 1 – (a) Dendrogram produced by average link method and according to L2-Wasserstein distance on the BRS distributions. (b) Estimated BRS probability density for each subject, where the dashed line positions the empirical cutoff of 3.0 ms/mmHg. The colored green subject group identified by cluster analysis (1a) corresponds to green colored BRS estimated probability density function with lowest BRS values and lowest variability (1b).

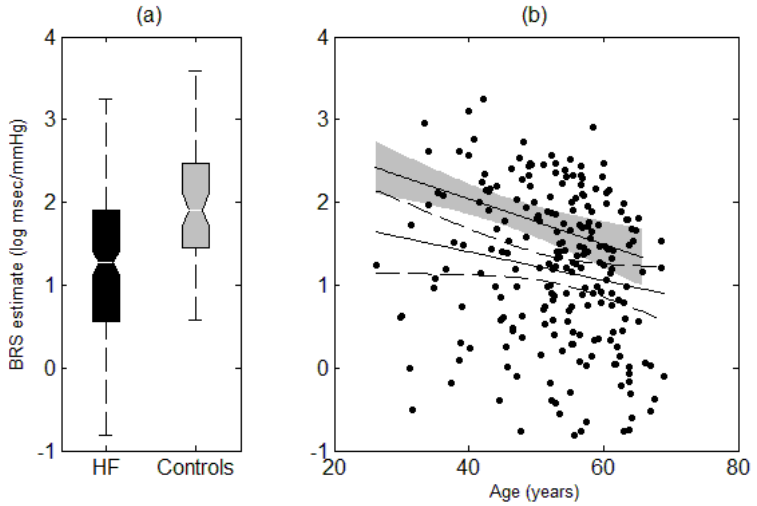

Figure 2 - Baroreflex sensitivity (BRS) in HF and in control groups: (a) boxplot of log BRS per group and (b) dispersion diagram age vs log BRS for HF group. In (a), the 95% CI for the group median is represented by the boxplot notch around the median. In (b), linear regression line with 95% CI is shown for HF and the shaded area defines the 95% CI for controls.

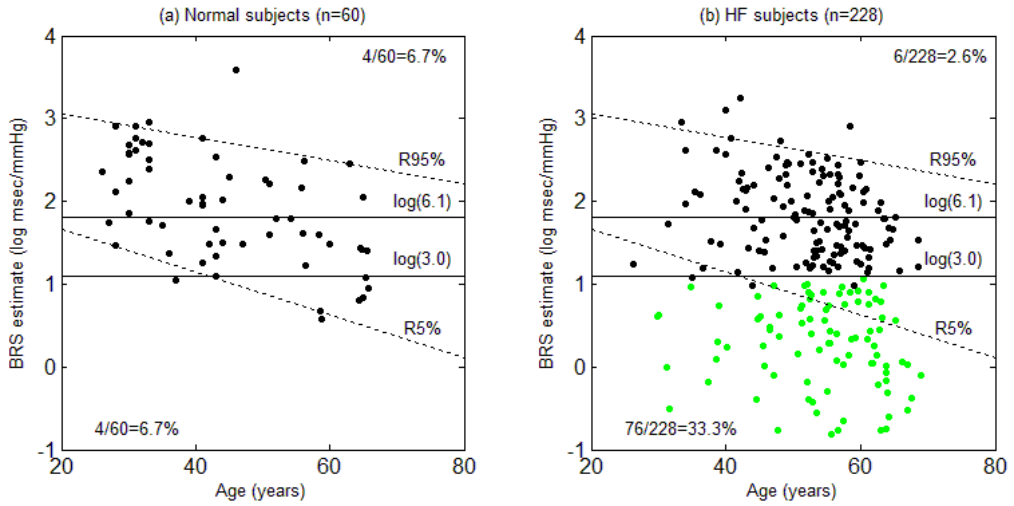

Figure 3 – Log BRS versus age in (a) controls and in (b) HF patients. The colors in (b) follow the clustering displayed in Figure 1. Full lines localize the empirical cutoffs 3.0 and 6.1 ms/mmHg and dotted lines localize R5% and R95% lines obtained from control subjects. Top and bottom statistics represent the percentage of subjects, respectively below R5% and above R95% for each group.

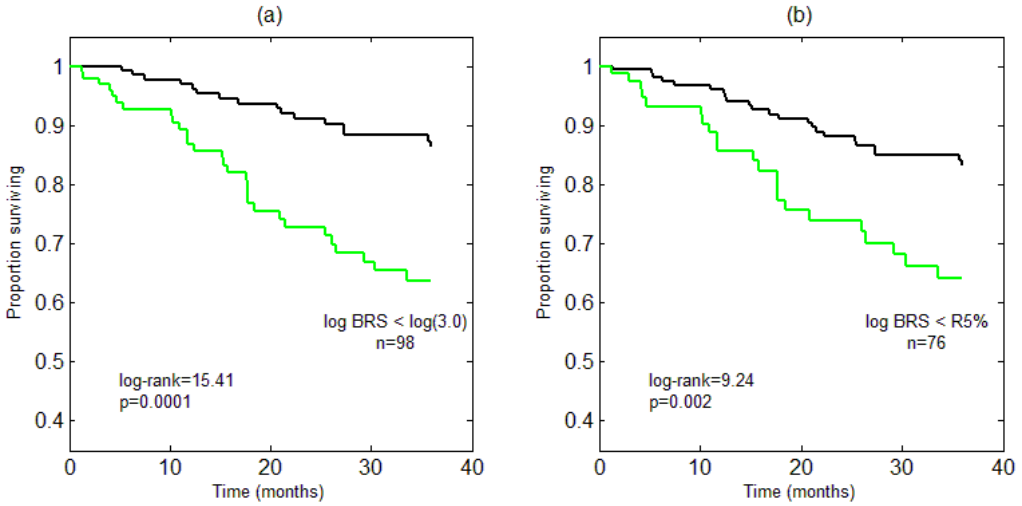

Figure 4 – Kaplan-Meier survival curves considering BRS dichotomization according to (a) log BRS < log(3.0) criterion and (b) log BRS < R5% criterion.

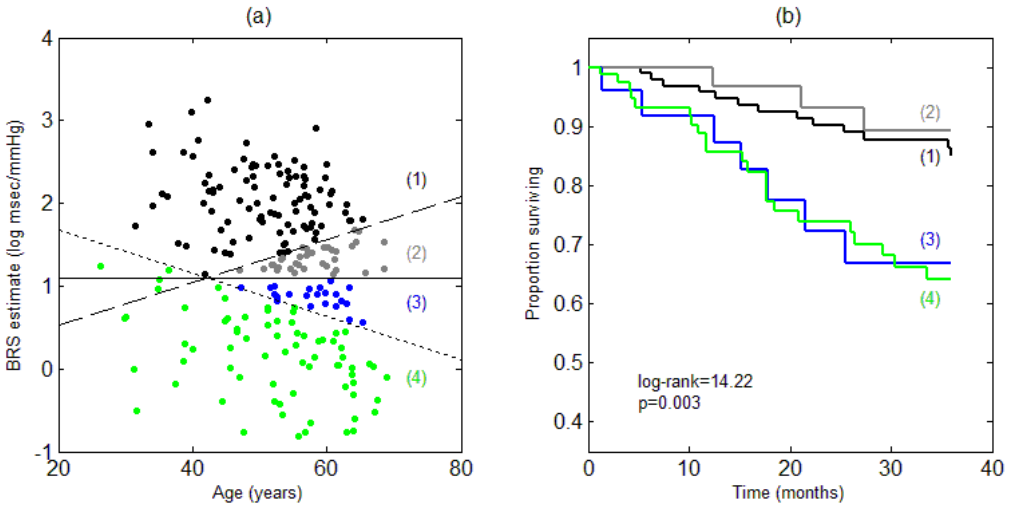

Figure 5 – (a) Log BRS versus age in HF group. Lines represent R5%, log(3.0) and R5% line mirrored by reflection at log(3.0) (dashed line). (b) Kaplan-Meier survival curves according to the regions colored in Figure (a). Pairwise comparisons indicate significant differences between all regions except for the pairs of regions (1,2) and (3,4) at 5% significance level.

Variable	HF	Control	p-value
# of subjects	228	60	
Log BRS	1.18±0.96	1.91±0.66	<0.0001
BRS (ms/mmHg) Geometric mean [95% CI]	3.25 [2.86,3.68]	7.78 [5.73,8.04]	
Log BRS and age regression	log BRS = –0.017*age+2.10, r= –0.17, F= 5.77, p<0.02	log BRS = –0.027*age+3.13, r=–0.53, F=22.70, p<0.0001	
Log BRS and age regression	log BRS = –0.022*age+2.844-0.562*(1-group), F=16.99, p<0.0001 (group=0 for HF and group=1 for controls)		
R5%	---	log BRS = -0.026*age+2.19	
R95%	---	log BRS = -0.014*age+3.34	
% subjects below R5%	76/228	4/60	<0.001
% subjects above R95%	6/228	4/60	0.13
% subjects below 3.0	98/228	7/60	<0.001

Table 1 – Results comparing HF and control groups.