Reproducibility of methods for assessing baroreflex sensitivity in normal controls and in patients with chronic heart failure

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ABSTRACT

Baroreflex sensitivity (BRS) conveys useful prognostic information in patients with heart disease, yet methods for its quantification suffer from poor reproducibility and test failure in some patients with heart failure. We set out to compare the short-term reproducibility and success rate of four different methods of assessing BRS in normal subjects and patients with chronic heart failure (CHF). A total of 31 patients with CHF and 18 normal controls underwent BRS testing using four techniques: (1) bolus phenylephrine (BRSPhe), (2) α-index in both low- and high-frequency bands (BRSαLF and BRSαHF respectively), (3) the sequence method (BRSSeq), and (4) a new 0.1 Hz controlled-breathing, time-domain analysis method (BRSCbr). Each subject underwent two test episodes with each method on the same day. The average values for BRS in patients and controls respectively were: BRSPhe, 4.4 (±4.4) ms/mmHg and 19.8 (±11.5) ms/mmHg; BRSαLF, 5.6 (±4.1) ms/mmHg and 15.4 (±5.0) ms/mmHg; BRSαHF, 7.1 (±7.0) ms/mmHg and 25.1 (±8.3) ms/mmHg; BRSSeq, 7.7 (±6.3) ms/mmHg and 22.5 (±8.4) ms/mmHg; BRSCbr, 6.6 (±5.9) ms/mmHg and 22.8 (±10.8) ms/mmHg. The coefficients of variation (S.D. of the difference in repeated values divided by mean) in patients and controls respectively were: BRSPhe, 85.6% and 52.2%; BRSαLF, 65.9% and 33.7%; BRSαHF, 99.7% and 52.1%; BRSSeq, 30.7% and 40.4%; BRSCbr, 30.7% and 19.6%. The numbers of test failures in patients were: BRSPhe, 15; BRSαLF, 7; BRSαHF, 5; BRSSeq, 14; BRSCbr, 1. Of the four techniques assessed for measuring BRS, the controlled breathing time-domain method yielded the best reproducibility and lowest failure rate in controls and in patients with CHF.

INTRODUCTION

Baroreflex sensitivity (BRS) is frequently impaired in patients with chronic heart failure (CHF) [1], recent myocardial infarction (MI) [2], hypertension [3] or obstructive sleep apnoea [4]. In addition, the degree of impairment of BRS provides prognostic information in CHF patients [5] and predicts cardiac mortality after MI [6]. The standard method of assessing BRS used in these prognostic studies involves the intravenous injection of a bolus of phenylephrine, which is used to elicit a transient increase in blood pressure. This increase in blood pressure is sensed by the baroreceptors and triggers a reflex decline in heart rate. The magnitude of the reflex change in heart rate is used to calculate BRS. The reproducibility of this method has been questioned, and alternative methods have been developed to improve its accuracy and reliability. In this study, we compared the reproducibility and success rate of four different methods of assessing BRS in normal subjects and patients with chronic heart failure (CHF). We used four techniques: (1) bolus phenylephrine (BRSPhe), (2) α-index in both low- and high-frequency bands (BRSαLF and BRSαHF respectively), (3) the sequence method (BRSSeq), and (4) a new 0.1 Hz controlled-breathing, time-domain analysis method (BRSCbr). Each subject underwent two test episodes with each method on the same day. The average values for BRS in patients and controls respectively were: BRSPhe, 4.4 (±4.4) ms/mmHg and 19.8 (±11.5) ms/mmHg; BRSαLF, 5.6 (±4.1) ms/mmHg and 15.4 (±5.0) ms/mmHg; BRSαHF, 7.1 (±7.0) ms/mmHg and 25.1 (±8.3) ms/mmHg; BRSSeq, 7.7 (±6.3) ms/mmHg and 22.5 (±8.4) ms/mmHg; BRSCbr, 6.6 (±5.9) ms/mmHg and 22.8 (±10.8) ms/mmHg. The coefficients of variation (S.D. of the difference in repeated values divided by mean) in patients and controls respectively were: BRSPhe, 85.6% and 52.2%; BRSαLF, 65.9% and 33.7%; BRSαHF, 99.7% and 52.1%; BRSSeq, 30.7% and 40.4%; BRSCbr, 30.7% and 19.6%. The numbers of test failures in patients were: BRSPhe, 15; BRSαLF, 7; BRSαHF, 5; BRSSeq, 14; BRSCbr, 1. Of the four techniques assessed for measuring BRS, the controlled breathing time-domain method yielded the best reproducibility and lowest failure rate in controls and in patients with CHF.
pressor agent (usually phenylephrine), as described by Smyth et al. in 1969 [7]. However, this technique is not without difficulties. There is a high test failure rate in patients with an attenuated BRS, and the intra-subject reproducibility is poor even in normal controls, with the original authors themselves remarking that [7] ‘the average values…conceal in some cases a considerable variation in sensitivity in what is ostensibly the same state of waking or sleep. The reasons for this are not entirely clear’. The bolus phenylephrine test is also invasive, requiring an intravenous injection. These problems, combined with the development of non-invasive methods of measuring blood pressure (BP), have resulted in the search for simpler measures of BRS.

Ideal qualities of a technique are speed, simplicity of execution, safety, non-invasiveness, a low failure rate in patient groups and, arguably the most important, a high degree of intra-subject reproducibility. Most studies of reproducibility of non-invasive measures of BRS have concentrated on healthy volunteers [8–10] rather than on patient groups. One study showed that BRS assessed by spectral analysis showed better reproducibility than that obtained following an infusion of phenylephrine, again in normal controls [11]. No studies to date have quantified the reproducibility of BRS measurements in a population of patients with CHF, where the BRS value may have important prognostic implications. Respiration has a significant effect on cardiovascular rhythms [12], but its effect on measures of BRS has been largely ignored [13]. It has been shown, however, that controlled respiration improves the correlation between the x-index in the respiratory band and BRS assessed by bolus phenylephrine [14]. We have developed a non-invasive measure of BRS that involves time-domain analysis of the interbeat interval (RR interval) and BP in subjects performing controlled respiration.

We set out to compare the standard phenylephrine technique and three non-invasive methods of BRS measurement (spectral analysis, the sequence method and the new controlled breathing protocol) in heart failure patients and normal controls. We assessed short-term reproducibility, failure rate and the agreement between techniques.

**METHODS**

**Subjects and measurements**

A total of 31 patients were recruited from a specialist CHF clinic. Exclusion criteria included: diabetes mellitus, atrial fibrillation or greater than 2 ventricular ectopic beats per min, the presence of a permanent pacemaker, contra-indications to phenylephrine administration, and clinical instability within the preceding 3 months. A group of 18 normal controls was also studied; these subjects had no significant past medical history and no abnormalities on examination, and were not taking regular medication. All subjects gave informed consent, and the study was approved by the local Ethical Committee.

The subjects were studied between 13.00 and 17.00 hours under standardized conditions, in a quiet room at a comfortable temperature. All fasted for at least 2 h before testing, and were not allowed to smoke or to drink alcohol- or caffeine-containing beverages for 24 h before the study.

On arrival at the investigation unit, a venous cannula was inserted into a forearm vein, and the subjects rested supine for 30 min. Each subject then underwent three investigations: (A) two successive 5 min recordings at rest; (B) two episodes of 5 min of controlled breathing separated by 5 min of rest; and (C) two episodes of boluses of phenylephrine. Two test episodes were therefore obtained in all subjects for each method of assessing BRS. The difference between the results from the two test episodes was used to calculate the short-term reproducibility of the method.

During each investigation, RR interval, BP and respiration were recorded. The procedures were performed in the same order in all subjects. The resting recording was performed first in case a period of controlled breathing caused excessive conscious awareness of respiration, altering the natural breathing pattern. The phenylephrine doses were given last to prevent any lasting effect of the cumulative drug dosage on the physiological reflex system being studied by the other techniques.

**Data collection**

BP was measured by a Finapres device (model 2300; Ohmeda), with the cuffed finger resting comfortably at the level of the heart. The Finapres cuff was wrapped around the index finger of the left hand. The subjects underwent several minutes of accustomization to the Finapres, and the servo-adjust mechanism was turned off prior to recording. The ECG was acquired from the limb lead with the largest R-wave (usually lead II). Respiratory rate was measured by impedance plethysmography. All data were sampled at 1000 Hz on a computer using an analogue-to-digital converter (National Instruments). The readings were saved on to floppy disk and analysed off-line with custom-designed software. The program measured RR intervals and beat-to-beat systolic pressure. Ectopic beats were corrected by interpolation.

**Phenylephrine method**

This was performed in accordance with the standard method originally described by Smyth et al. [7], using phenylephrine instead of angiotensin and non-invasive BP measurement [15]. Phenylephrine was injected as a
Reproducibility of measures of baroreflex sensitivity

Figure 1  Assessment of BRS using time-domain analysis during controlled breathing
In each case, the broken line shows the measured signal and the continuous line shows the filtered signal.

Table 1  Failure rate and BRS values in patients and controls
BRS values are means (S.D.).

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 31)</th>
<th>Controls (n = 18)</th>
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<tr>
<td></td>
<td>BRS (ms/mmHg)</td>
<td>No. of failures</td>
</tr>
<tr>
<td>BRS_{Phe}</td>
<td>4.4 (4.4)</td>
<td>15/62</td>
</tr>
<tr>
<td>BRS_{LF}</td>
<td>5.6 (4.1)</td>
<td>7/62</td>
</tr>
<tr>
<td>BRS_{HF}</td>
<td>7.1 (7.0)</td>
<td>5/62</td>
</tr>
<tr>
<td>BRS_{Seq}</td>
<td>7.7 (6.3)</td>
<td>14/62</td>
</tr>
<tr>
<td>BRS_{bcr}</td>
<td>6.6 (5.9)</td>
<td>2/62</td>
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</table>

bolus at a starting dose of 2 μg/kg; this was increased by 50 μg per bolus until the rise in systolic BP (SBP) was at least 15 mmHg. The bolus injection was repeated at least three times at the adequate dose, and the linear regression slope of RR interval and SBP was calculated for each bolus. The final result (BRS_{Phe}) for each episode was computed as the mean of three values. Each subject underwent two test episodes.

Spectral analysis
Each 10 min period of rest was broken up into two 5-min sections, which were analysed separately by a previously published method [16]. Power spectral analysis was performed on the RR interval and SBP data through the use of an autoregressive algorithm [17], with the model order selected according to the Akaike information criterion; a model order between 12 and 18 was considered adequate in all cases. The following components were considered: low-frequency (LF) power in the 0.04–0.15 Hz band, and high-frequency (HF) power in the 0.15–0.4 Hz band. The α-index was computed as the square root of the ratio between RR interval and SBP spectral powers in the two major bands of LF (αLF) and HF (αHF), in the presence of an adequate coherence (0.5) between the RR interval and SBP, as assessed by cross-spectral analysis [16].

Sequence method
The same two 5-min sections of rest were also used for sequence method analysis [9]. The time series of RR interval and SBP were scanned to identify the sequences in which RR and SBP increased or decreased concurrently over 3 or more beats. The minimum change required was 1 mmHg for SBP and 4 ms for RR interval. The linear correlation between RR interval and SBP was computed for each sequence. The regression slope was calculated in those sequences with correlation coefficients of > 0.8. The average value of the individual slopes occurring within the 5 min episode was taken as BRS_{Seq}.

The results for BRS obtained from rises in SBP were combined with those obtained from falls in SBP, as it has been shown previously that the values are consistent [14].

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Controlled breathing protocol

Subjects were guided to breathe at 0.1 Hz by following a sinusoidal visual and auditory signal. Heart rate, BP and respiration were measured as described above. Each subject was initially observed by an investigator for 30 s to ensure that the breathing pattern was being followed adequately before recording began. Two 5 min episodes were recorded. The resultant RR interval and SBP signals were processed with a simple time-domain digital filter to extract the signal component at the frequency of interest (0.1 Hz), as shown in Figure 1. BRS\textsubscript{Cbr} was calculated as the ratio of the average amplitude of oscillations in RR interval to the average amplitude of oscillations in SBP.

Statistical analysis

The data are expressed as means ± S.D. Measures of reproducibility were calculated according to the method of Bland and Altman [18], which involves plotting the

Figure 2  Relationship between the difference for the two test episodes and the mean of the test episodes for (a) bolus phenylephrine, (b) αLF, (c) αHF, (d) sequence method and (e) controlled breathing protocol
signed difference between two measures against the mean of the two measures. The intra-subject reproducibility between the two test episodes was given as the coefficient of variation (S.D. of difference between two BRS values divided by mean of the BRS values) and the limits of reproducibility (mean of the difference between two BRS values ± 2 · S.D. of difference between BRS values), which indicates the range containing 95% of differences.

RESULTS

Characteristics of patients and controls
A total of 31 patients were studied. Their average age was 62 years (S.D. 12 years; range 25–83 years). Of these, 20 patients had heart failure due to coronary artery disease; the other 11 had dilated cardiomyopathy. The mean ejection fraction was 27% (S.D. 10%). With regard to medication, 29 of the patients were taking an angiotensin-converting enzyme (ACE) inhibitor, 13 were on a nitrate preparation, 27 were receiving diuretics, and none was receiving a β-blocker. Twenty-nine of the patients were male and two were female. The normal control group consisted of 13 males and 5 females, with an average age of 32 years (S.D. 13 years; range 20–69 years).

The mean values obtained for BRS using the different methods are shown in Table 1. Where two BRS values were obtained successfully using a method, their mean was taken as the value for that subject. If one of the two test episodes failed to provide a result, the single value from the successful episode was used. The numbers of episodes failing to give a result are also shown in Table 1. There were more test failures in the patient group than in the normal controls. Failures occurred with the phenylephrine method because of an inability to raise SBP by 15 mmHg in several of the patient group and due to marked bradycardia in one of the control group. Failures with the spectral analysis method were due to inadequate coherence between RR interval and SBP. The sequence method failed where there were no sequences, during a 5 min recording period, where the SBP increased or decreased for 3 or more consecutive beats. This problem was encountered frequently in the patient group. The single patient who failed with the controlled breathing protocol was unable to match his breathing to the target for 5 min.

Measurement of reproducibility
For each BRS technique, short-term reproducibility was quantified using results from those subjects for whom two values were obtained. The Bland–Altman plots for each BRS method are shown in Figures 2(a)–2(e).

The limits of reproducibility (the range within which 95% of the intra-method differences lie) were smaller for patients than for controls. This is due, in large part, to the smaller mean BRS values in patients than in controls. The coefficient of variation was therefore calculated, which expresses the S.D. of the difference as a proportion of the mean value. The results for patients and controls for each method are shown in Table 2.

BRS_HF and BRS_Phe showed the poorest reproducibility in both patients and controls. In patients, the most reproducible methods were the controlled breathing protocol and the sequence method (coefficient of variation 30.7% in both). In the control group the most reproducible methods were the controlled breathing protocol (coefficient of variation 19.6%) and the x-index in the LF band (coefficient of variation 33.7%).

Agreement
Table 3 shows the correlation matrix of the different methods. Results from patients and controls have been combined. The inter-method correlation coefficients must be viewed in the context of the correlation

| Table 2 | Reproducibility of tests of BRS in patients and controls |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| BRS_Phe         | 85.6            | — 6.0 to 9.1 (n = 17) | 52.2            | — 21.7 to 19.7 (n = 17) |
| BRS_LF          | 65.9            | — 6.7 to 8.1 (n = 24) | 33.7            | — 10.9 to 9.8 (n = 16) |
| BRS_HF          | 99.7            | — 14.4 to 14.9 (n = 27) | 52.1            | — 31.5 to 20.9 (n = 17) |
| BRS_seq         | 30.7            | — 5.1 to 4.4 (n = 21) | 40.4            | — 21.2 to 15.2 (n = 16) |
| BRS_con         | 30.7            | — 3.8 to 4.4 (n = 30) | 19.6            | — 9.3 to 8.6 (n = 18) |

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coefficients of each method with itself, obtained by comparing the two episodes for each subject (Table 4). It is noteworthy that these coefficients are almost all distinctly less than 1, with the exception of that for BRS<sub>Cbr</sub>.

### Table 4 Correlation between two independent test episodes for each technique

<table>
<thead>
<tr>
<th>Method</th>
<th>Correlation of episode 1 with episode 2</th>
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<tbody>
<tr>
<td>BRS&lt;sub&gt;Phe&lt;/sub&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>BRS&lt;sub&gt;zLF&lt;/sub&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>BRS&lt;sub&gt;SX&lt;/sub&gt;</td>
<td>0.68</td>
</tr>
<tr>
<td>BRS&lt;sub&gt;seq&lt;/sub&gt;</td>
<td>0.03</td>
</tr>
<tr>
<td>BRS&lt;sub&gt;Cbr&lt;/sub&gt;</td>
<td>0.96</td>
</tr>
</tbody>
</table>

### DISCUSSION

This study compared the same-day reproducibility of several methods of measuring BRS in patients with CHF and in normal controls. The ‘gold-standard’ method of measuring BRS (phenylephrine bolus) performed badly in both patients and controls. It was poorly reproducible and suffered from the highest failure rate. The z-index in the HF band also performed badly, with similarly poor reproducibility. The x-index in the LF band showed good reproducibility in the control group, but not in the patient group. Conversely, the sequence method was reproducible in the patient group, but less so in the controls. However, this method had the second highest failure rate of all the methods in the patient group. The controlled breathing protocol had the lowest failure rate and also the best reproducibility in both subject groups.

No previous study has quantified the reproducibility of all of these methods of BRS measurement in a group of patients with CHF. Lord et al. [11] assessed the withinday and within-week reproducibility of BRS in 26 normal controls using the Valsalva manoeuvre, spectral analysis in the LF and HF bands and an infusion of phenylephrine. They found that zLF was the most reproducible technique (coefficient of variation 25%; range 3.5–42.4%) and the Valsalva manoeuvre the worst (coefficient of variation 29.3%; range 13.8–93.1%). The highest number of test failures occurred with the phenylephrine method. Dawson et al. [10] compared the Valsalva manoeuvre, spectral analysis and the sequence method in 39 normotensive controls from within a wide age range performed 1 week and 6 months apart. In contrast with the previous study [11], they found that the Valsalva manoeuvre gave the best reproducibility (coefficient of variation 16.8%), although it had the highest failure rate. Herpin and Ragot [8] evaluated the mid- and long-term (1 year) reproducibility of the sequence method and of spectral analysis in 14 healthy volunteers. They found high reproducibility with both techniques, which was improved further if the measurements were performed with the subject standing. Iellamo et al. [9] looked at the reproducibility on consecutive days of the sequence method in 24 healthy volunteers in the supine and standing positions, undergoing static hand-gripping and performing mental arithmetic. The coefficients of variation ranged from 15.0 to 19.7%, with no significant effects of the various stimuli. Other studies have compared the non-invasive measures of BRS with the phenylephrine technique in normal controls and in patients post-MI or with hypertension [14,19–21]. The correlation coefficients obtained in these studies were similar to the ones we obtained in the present study, ranging from 0.5 to 0.64 for the sequence method and from 0.48 to 0.63 for the spectral method. Although this may appear to indicate that the physical processes being studied are distinct from each other, there is an alternative explanation. If the reproducibility of one method is less than perfect, then the correlation coefficient of two successive measurements by the same method will be not 1 (i.e. perfect), but considerably less [22]. This places a ceiling on the maximum possible correlation coefficient between one measure of BRS and another. The situation is even more severe if the second technique also has imperfect reproducibility. Thus poor correlation with an old technique cannot be used as a reason for dismissing a new technique, unless the old technique has excellent reproducibility [22].

A large contribution to the poor reproducibility of BRS measurements may arise from the effect of respiration. Varying respiratory rates and depths can have major effects on BP and RR interval. As the respiratory rate increases, the RR interval and arterial pressure spectral power decline significantly [23] at both respiratory and low frequencies [13]. In addition, at a fixed respiratory rate, RR interval power at the respiratory frequency is significantly greater at a tidal volume of 1500 ml than at 1000 ml [13]. As a result, the value obtained for BRS with methods that utilize RR interval power at different frequency bands may well depend on the respiratory rate and depth of the subject being tested. If these were to differ between test episodes, then the reproducibility of the test would be impaired. This may help to explain the difference seen between patients and controls with the z-index in the LF band. Control subjects have a slow, regular rate of respiration at rest which does not vary greatly over time. This leads to temporal stability of the power spectra of RR interval and SBP, and hence to better reproducibility. In heart failure and post-MI, respiratory frequency is generally higher and is more irregular over time. Coherence is, therefore, lower between RR interval and SBP, and consequently there are more test failures and poorer test reproducibility. In our study, the mean spontaneous respiratory rate of the patients with CHF was 17.6
breaths/min, and that of the normal controls was 11.6 breaths/min. Irregular, or differing, respiratory rates between test episodes may also affect the BRS results obtained by the sequence method and the phenylephrine method, thus worsening reproducibility. In addition, phenylephrine activates baroreceptor pathways independently of its pressor effects. Specifically, it activates \( \alpha \)-adrenergic receptors in the smooth muscle of the aortic arch and carotid sinus, which increases baroafferent discharge and sensitizes baroafferent responses to changes in pressure [24, 25].

Furthermore, phenylephrine produces direct effects on pre-ganglionic and post-ganglionic cardiac parasympathetic nerves which may alter the derived index of BRS [26,27]. The two experimental groups were not age-matched, and so the differences in reproducibility may be age-related rather than disease-related. Although the absolute value of BRS declines with age, the reproducibility of non-invasive measures of BRS in control subjects does not [10].

The unwanted effect of respiration is removed in the controlled breathing protocol, as all subjects breathe at the same rate. Previous work has shown that subjects automatically regulate their tidal volume when the rate is entrained to an external rhythm [23]. Consequently, it is not necessary explicitly to control the subjects’ tidal volume, which permits a relatively simple protocol. This technique requires subjects to breathe at 0.1 Hz, because this is the prime natural resonant frequency of the RR interval/SBP system [28]. Stimulating breathing at this frequency yields single, clear, coherent oscillations in both RR interval and SBP [23]. Another advantage with the new technique is that it is unsophisticated and transparent, unlike frequency-domain analysis by autoregressive methods. Finally, BRS assessment by the phenylephrine and sequence methods relies on an invariant time delay between BP and RR interval across all subjects. In reality, the time delay may differ between subjects and subject groups. The proposed time-domain analysis focuses on amplitude relationships which can be measured independently of time delay. Although a possible criticism of this method is that it may not be measuring a ‘pure’ arterial baroreceptor reflex, this criticism could equally be levelled at any of the measures of BRS. All non-invasive methods involve not only the pure arterial baroreflex but also other cardiovascular and thoracic stretch reflexes; the phenylephrine bolus technique, while it may do this less, additionally involves other unquantified pharmacological effects.

The sequence method had a high failure rate in the patient group, which, despite the good reproducibility of this method, would appear to limit its use in clinical practice. Several of our patients exhibited subtle but consistent pulsus alternans, which precluded the existence of 3 beats where the SBP rose (or fell), thus causing failure of the technique. In contrast, the controlled breathing technique will produce a result in any subject who is able to follow the breathing protocol. Our study assessed the same-day reproducibility of the techniques. This avoided the possibility of significant changes in clinical state in the patient group, which could artefactually reduce reproducibility.

In conclusion, we have shown that BRS measurement using a controlled breathing protocol and a simple time-domain analytical technique is easy to perform (taking only 5 min of the patient’s time and 30 s of analytical time), and has the highest reproducibility and the lowest failure rate. These findings would seem to commend it as a method for following serial changes in BRS in both patients and control subjects.

**REFERENCES**


Received 14 April 1999/24 May 1999; accepted 12 July 1999