Effect of altering conditions of the sequence method on baroreflex sensitivity
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Background The sequence method is widely used as a simple, non-invasive measure of baroreflex sensitivity (BRS). This technique, originally described in anaesthetized cats, has been transferred virtually unchanged to humans, without evidence that the optimal values in cats are the same as those in patients with cardiovascular disease.

Objective To study the effect of altering the components of the sequence method on the measured BRS in patients with chronic heart failure (CHF) and in normal individuals.

Methods Eighty patients with CHF [aged 62 ± 12 years (mean ± SD)] and 40 normal control individuals [aged 38 ± 15 years (mean ± SD)] underwent measurement of heart rate and non-invasive blood pressure. Altering only the shift between blood pressure and R–R interval and the required correlation coefficient of the regression line had no effect on the value of BRS, but had a significant effect on the number of valid sequences. Alteration of the blood pressure or R–R interval thresholds, however, affected not only the number of valid sequences, but also the value of BRS in both groups. In normal controls, agreement with the bolus phenylephrine method was improved by increasing the blood pressure threshold, although this led to a reduction in the number of valid sequences. In patients with CHF, agreement was optimized by decreasing both the blood pressure and R–R interval thresholds. This also had the effect of increasing the number of valid sequences.

Conclusion Changes should be made to this technique, to optimize its validity in conscious humans, particularly when applied to patients with attenuated BRS. J Hypertens 19:1279–1287 © 2001 Lippincott Williams & Wilkins.

Introduction Attenuation of baroreflex sensitivity (BRS) predicts poor outcome after myocardial infarction and in patients with chronic heart failure [1–3]. The first measurements of BRS in humans [4] were performed by intravenous injections of a pressor agent that increases blood pressure (measured intra-arterially), which in turn reduces heart rate. Non-invasive techniques have subsequently been developed, but acceptance of these new techniques has been limited by their relatively poor agreement with the ‘gold-standard’ invasive method [5].

The spontaneous, or sequence method, first described in 1985 [6] is widely used as a measure of BRS. R–R interval and beat-to-beat systolic blood pressure data are scanned and sequences of three or more beats in which the blood pressure and R–R interval concomitantly increase (or decrease) by more than a threshold value are identified. The BRS is defined as the slope of the regression line between the data points in these sequences. This technique was initially developed in anaesthetized cats [6,7]. However, the R–R interval and blood pressure thresholds, blood pressure to R–R interval shift and threshold correlation coefficient of the regression line involve essentially arbitrary constants, which have been adopted virtually unchanged for the study of humans. There is no evidence that these are the optimal values.

If the arbitrary constants used in the standard sequence method are not optimal, the results from the technique may have been unnecessarily poor. In order to optimize the method, by identifying optimum values for these constants, we assessed the effect on the BRS, derived by the sequence method, of altering the R–R interval and blood pressure thresholds, the blood pressure shift and the regression line correlation coefficient, in healthy control individuals and patients with chronic heart failure, and compared them with other conventional methods of measuring BRS.
Methods
Participants and measurements
Eighty patients with chronic heart failure were re-
cruited from a specialist clinic. They were diagnosed
on the basis of clinical assessment (a history of dysp-
noea and symptomatic exercise intolerance, with pre-
vius signs of pulmonary congestion or peripheral
oedema), evidence of left ventricular dysfunction from
radionucleide ventriculography or echocardiography, or
both. Patients with atrial fibrillation, permanent pace-
makers, more than two ectopic beats per minute or
clinical instability within the preceding 3 months were
not eligible for the study. Forty normal control indivi-
duals were also studied; they had no significant past
medical history, no abnormalities on examination, and
were not taking regular medication. All participants
gave informed consent and the study was approved by
the local ethics committee.

The individuals were studied between 1300 h and
1700 h, under standardized conditions, in a quiet room
at a comfortable temperature. All were fasted for at
least 2 h before testing and were not allowed to smoke
or drink alcohol- or caffeine-containing beverages for
24 h before the study.

On arrival to the investigation unit, the participants
rested supine for 30 min and then underwent a 10 min
recording of heart rate and non-invasive measurement
of blood pressure. Twenty-six patients and 15 controls
also underwent assessment of BRS by the bolus pheny-
lephrine method [4].

Data collection
Blood pressure was measured non-invasively by a
Finapres device (model 2300; Ohmeda, Louisville,
California, USA), with the cuffed finger resting comfor-
tably at the level of the heart. The Finapres cuff was
wrapped around the index finger of the left hand. The
participants underwent several minutes of accustomiza-
tion to the Finapres and the servo-adjust mechanism
was turned off before recording. The electrocardiogram
was acquired from the limb lead with the largest R
wave (usually lead II). All data were sampled at
1000 Hz on a computer using an analog-to-digital con-
verter (National Instruments, Newbury, Berkshire).
The readings were saved onto floppy disk and analysed
off-line with custom software, which measured R–R
intervals and beat-to-beat blood pressure.

Baroreflex sensitivity
BRS by the sequence method
The time series of R–R interval and systolic blood
pressure (SBP) were scanned to identify the sequences
in which R–R and SBP concurrently increased or
decreased over three or more beats. The standard
thresholds for SBP and R–R interval are 1 mmHg and
4 ms, respectively. In this study, we varied the thresh-
hold for SBP from 0 to 2 mmHg and the R–R interval
threshold from 0 to 6 ms. In the standard version of the
sequence method, there is a shift of +1 between the
blood pressure pulse and the R–R interval, with the
blood pressure pulse being plotted against the following
R–R interval. In this study, we assessed a blood
pressure shift from −4 to +4 with respect to the R–R
intervals (Fig. 1).

The linear correlation between R–R and SBP was
computed for each sequence. In the standard method,
the sequence is only considered valid if the correlation
coefficient is > 0.80. In this study, several thresholds of
correlation coefficient were compared: 0, 0.8, 0.95, 0.96,
0.97, 0.98, 0.99 and 1.0. The average value of the
individual slopes of the valid sequences was taken as
the BRS.

BRS by the bolus phenylephrine method
This was performed according to the standard modifica-
tion of the method of Smyth et al. [4], which uses
phenylephrine instead of angiotensin and non-invasive
blood pressure measurements instead of intra-arterial
ones [8]. The procedure was carried out after informed
consent and with appropriate resuscitation equipment
and staff available, in 26 patients and 15 normal
individuals. Phenylephrine was injected as a bolus at a
starting dose of 2 µg/kg, increasing by 50 µg until the
increase in SBP was at least 15 mmHg. The bolus

![Diagram showing the shifts between blood pressure (BP) and R–R interval. ECG, electrocardiogram.](image-url)
injection was repeated at least three times at the adequate dose and the linear regression slope of R–R and SBP was calculated for each bolus. The final result for each episode was computed as the mean of at least three values.

**BRS by the spectral analysis method**

BRS was calculated by the spectral analysis method as previously described. Briefly, power spectral analysis was performed on the same R–R interval and SBP data used for the sequence method, through the use of an autoregressive algorithm [9], with the model order selected according to the Akaike information criterion [10]. A model order between 12 and 18 was found to be appropriate in all cases. The component in the low-frequency band (0.04–0.15 Hz) was considered. The α-index was computed as the square root of the ratio between R–R and systolic blood pressure spectral powers in the low-frequency band, in the presence of an adequate coherence (> 0.5) between the R–R interval and SBP as assessed by cross-spectral analysis [5,6].

**Statistical analysis**

Numerical distributions are described by their mean ± SD. Comparisons between group means were carried out with Student’s *t*-test and, in the case of multiple comparisons, analysis of variance (ANOVA).

**Results**

**Patient characteristics**

The average age of the 80 patients was 62 ± 12 years. Fifty-three had heart failure due to coronary artery disease, 25 had idiopathic dilated cardiomyopathy and two had heart failure due to mitral regurgitation. The mean ejection fraction was 0.30 ± 0.14. Eleven patients were in New York Heart Association class I, 46 in class II, 23 in class III. Seventy-four of the patients were taking an angiotensin converting enzyme inhibitor or angiotensin II receptor antagonist, 70 were taking diuretics, and none was receiving a β-blocker. Sixty-two of the patients were men and 18 were women. The average age of the 40 normal individuals was 38 ± 15 years. Twenty-five were men and 15 were women.

**Effect of changing blood pressure shift on measurement of BRS**

The effect of changing the blood pressure shift on the number of valid sequences is shown in Figure 2. For this analysis, the blood pressure threshold was set at 1 mmHg, the R–R interval threshold at 4 ms and the correlation coefficient threshold at 0.8. In both groups, there was a significantly larger proportion of valid sequences (number of valid sequences divided by total duration of recording multiplied by 100) when there was a zero shift between the blood pressure pulse and R–R interval (*P* < 0.0001 in controls and patients).

The effect of different blood pressure shifts on the value of BRS obtained is shown in Figure 3. Again, the maximal value for BRS was obtained with a zero blood pressure shift, but there was no significant difference in the values obtained within the two groups (*P* = 0.9 in patients and *P* = 0.1 in controls).

**Effect of changing sequence correlation threshold on measurement of BRS**

The effect of changing the threshold for the correlation coefficient on the number of valid sequences is shown in Figure 4. The blood pressure and R–R interval thresholds remained unaltered from before, and the blood pressure shift was set to the conventional 1 beat. As the threshold correlation coefficient was increased, there was a large decrease in the number of valid sequences in both patients and controls (*P* < 0.002 in both groups). The largest number of valid sequences...
occurred when the threshold coefficient was 0, but there was no significant difference between the number of sequences when the correlation coefficient was 0.8 ($P \approx 0.9$ for both groups).

Changing the required correlation coefficient had no effect on the value obtained for BRS (Fig. 5; $P = 0.9$ for both groups).

**Effect of changing blood pressure and R–R interval thresholds on BRS**

The effect of changing the blood pressure and R–R interval thresholds on the number of valid sequences is shown in Figure 6, with a blood pressure shift of 0, and a correlation coefficient threshold of 0.8. In patients with heart failure, increasing either the blood pressure threshold or R–R interval threshold resulted in a decrease in the number of valid sequences. However, in the healthy controls, an increase in R–R interval threshold (up to 6 ms) had very little effect on the number of valid sequences.

The effect of different thresholds on the value of BRS is shown in Figure 7. BRS measurement was highly sensitive to changes in the selected thresholds for blood pressure and R–R interval. In patients (Fig. 7a), the average measured BRS increased from 6.2 to 12.5 ms/mmHg as the blood pressure threshold was decreased from 2 to 0 mmHg (with an R–R interval threshold of 4 ms). The average measured BRS increased from 7.0 to 10.6 ms/mmHg as the R–R interval threshold was increased from 0 to 6 ms (with a blood pressure threshold of 1 mmHg). In controls (Fig. 7b), there was a marked dependence of measured BRS on blood pressure threshold, with the mean BRS increasing from 15.2 to 29.4 ms/mmHg as the blood pressure threshold decreased from 2 to 0 mmHg (with an R–R interval threshold of 4 ms). However, there
was little dependence of the measured BRS on R–R interval threshold.

**Comparison with other measures of BRS**
The BRS values obtained by changing the blood pressure and R–R interval thresholds were compared with BRS values obtained by the bolus phenylephrine and spectral analysis methods. The correlation coefficients are shown in patients (Fig. 8) and normal individuals (Fig. 9). It can be seen that, in both groups, the agreement between the sequence method and the other methods is highly dependent on the choice of blood pressure and R–R interval thresholds. The standard choice of thresholds (1 mmHg and 4 ms) did not result in the maximum agreement for BRS between the sequence method and the phenylephrine method or \( \alpha \)-index, in either groups.

**Discussion**
The development of risk stratification techniques for patients after myocardial infarction and for patients with chronic heart failure offers the potential to identify those at greatest risk, so that expensive (or risk-laden) diagnostic and therapeutic options can be targeted at those with most to gain. The arterial baroreflex has long been recognized to be attenuated in these patients [1–3], and the degree of this attenuation has been confirmed as an important risk stratifier, independent of
conventional clinical markers in patients after acute myocardial infarction [11]. The gold-standard method of measuring BRS, the bolus phenylephrine method, is invasive, which has limited its widespread use in clinical practice. The sequence, or spontaneous, method of measuring BRS is more widely used because it is non-invasive and, unlike the spectral α-index method, does not require the use of complicated and opaque
mathematics. However, the conventional sequence method approach has a relatively high test failure rate in patients with attenuated BRS [12], and has been reported to correlate only poorly with the bolus phentolamine method [5].

In this study, we have shown that altering the conditions for acceptance of valid sequences can have profound effects, not only on the number of valid sequences, but also on the value of BRS and hence its correlation with other measures of BRS. The individual components of the sequence method that we assessed in this study were the shift of blood pressure with respect to R–R interval, the threshold correlation coefficient of the regression line between blood pressure and R–R interval, and the blood pressure and R–R interval thresholds.

**Blood pressure shift**
In the original description of the sequence method [6], the shift between blood pressure and R–R interval was set at 1 beat, with the R–R intervals being paired with the systolic pressure peak occurring within the preceding R–R interval (Fig. 1). However, previous work with bolus injections of phentolamine [13] showed that, when blood pressure is transiently disturbed, the temporal relationship between the R–R interval sequence and the systolic blood pressure sequence depended on the heart rate, with a shift of 0 being optimal for individuals with heart rates of 75 beats/min or less and +1 for those with faster heart rates. Others have used the shift that produced the largest number of valid sequences (in most cases a shift of 0) [14,15]. In our study, altering the shift between −4 and +4 had a profound influence on the number of valid sequences obtained both in patients with heart failure and in normal controls. In both groups, the shift that gave the greatest number of valid sequences was 0. In our study, the mean heart rate of the patients with heart failure was $76 \pm 14$ beats/min and the mean heart rate of the normal controls was $66 \pm 10$ beats/min. However, the value for BRS was unaffected by the blood pressure shift in both groups, as described previously [15]. In the control group, the value of measured BRS peaks at blood pressure shifts of zero and +1, which may represent ‘true’ baroreflex sequences. The valid sequences obtained at the other blood pressure shifts correspond to the relative change between R–R interval and blood pressure from other modulatory systems. In patients with CHF, BRS is attenuated and so other cardiovascular reflexes (such as the chemoreflex) play a more prominent role in blood pressure and R–R interval control. As a result, the relationship between the changes in blood pressure and R–R interval is less dependent on their shift. It would seem optimal, therefore, to use the blood pressure shift that produces the
largest number of sequences, as this maximizes the opportunity to measure the BRS successfully, and does not bias the overall result.

Correlation coefficient threshold
Studies investigating BRS by the sequence method have used different threshold correlation coefficients to select valid sequences of beats, ranging from no specified threshold correlation coefficient [16] to a correlation coefficient threshold of more than 0.95 [17]. In this study, it can be seen that an increase in the threshold correlation coefficient results in a small decrease in the number of valid sequences, but has no effect on the value of BRS obtained. In fact, the number of sequences obtained with a threshold of 0.8 was not significantly different from that obtained with no correlation coefficient requirement. This is because the initial selection of sequences of three successive points with blood pressure and R–R interval moving consistently in the same direction results in sequences that have very high correlation coefficients (nearly all greater than 0.90). Therefore, excluding sequences with lower correlation coefficients has little impact on the number of valid sequences.

We suggest, therefore, that it would be optimal to place no condition at all on the correlation coefficient, as this maximizes the number of valid sequences without introducing bias into the BRS value.

Blood pressure and R–R interval thresholds
The original description of the sequence method in cats included sequences in which the blood pressure increased or decreased by 1 mmHg and the pulse interval either lengthened or shortened [6]. In a fuller description of the technique, the same authors proposed thresholds of 1 mmHg in blood pressure and 4 ms in R–R interval [7]. These thresholds are now very widely used in clinical research, although alternative values have ranged from 6 ms [18] down to no threshold [14].

The findings in our study are that altering the blood pressure and R–R interval thresholds has a profound effect, not only on the number of valid sequences, but also the measured value of BRS. In the healthy control group, alteration of the R–R interval threshold had no effect on the number of valid sequences, the value of BRS or the correlation between either the bolus phenylephrine or spectral analysis BRS. As these individuals have a normal BRS, therefore, it is not necessary to include an R–R interval threshold.

The situation is, however, very different in those with attenuated BRS, such as the patients in this study who had CHF. In this group, there is a much smaller increase in R–R interval for a given increase in blood pressure and therefore alterations in the R–R interval threshold will have an impact on the number of valid sequences. This is clearly seen in Figure 6a where, as both thresholds increase, the proportion of valid sequences decreases. The measured value of BRS itself increases as the blood pressure threshold decreases and the R–R interval threshold increases. Measured BRS is effectively the change in R–R interval divided by the blood pressure change. Therefore, if the R–R interval threshold is high and the blood pressure threshold is low, the validity thresholds will select sequences which yield a high measured BRS. These sequences are rare in patients with a low BRS and may represent artefact, rather than true baroreflex phenomena.

Agreement between sequence and other methods of measuring BRS
In the healthy controls, the correlation coefficients increased with increasing blood pressure threshold, although there was a slight decrease in correlation coefficient with the phenylephrine method at the greatest blood pressure threshold. The agreement between the different methods could therefore be optimized by increasing the blood pressure threshold above the standard of 1 mmHg, although this would result in a decrease in the number of sequences. Doing this would remove the small, artefactual, sequences that are not baroreflex-mediated. In patients with attenuated BRS, the method with the largest body of evidence for its prognostic usefulness is the bolus phenylephrine technique. Agreement between non-invasive measures of BRS and the phenylephrine technique is poor and this has led some authors to conclude that ‘the results obtained by means of non-invasive BRS assessments should not be used in clinical practice as an alternative to those obtained by the phenylephrine method’ [5]. However, against this point of view, several arguments can be offered. First, the reproducibility of the bolus phenylephrine method in patients with low BRS is far from excellent [12], which places an upper limit on how closely it can agree with any other method [19]. Second, it is likely that the phenylephrine method and sequence method do not assess the same part of the baroreflex arc and would therefore not be expected to agree. The changes in blood pressure are greater and sustained for longer with the phenylephrine method and may therefore explore the saturation point of the baroreceptors. In addition, the choice of an increase in blood pressure of 15 mmHg and a blood pressure shift of 1 beat are as arbitrary as the choices of threshold in
the sequence method, and phenylephrine may have direct pharmacological actions, unrelated to its effect on the baroreceptors. However, it can be seen that altering the thresholds in the sequence method also has an effect on agreement with the α-index BRS, which is calculated from the same blood pressure and R–R interval data, although this is more profound in the normal controls.

**Study limitations**

The patients with heart failure and healthy controls were not age-matched and it is possible that normal elderly patients with a depressed BRS will show a pattern similar to that in the heart failure group.

**Conclusion**

Modification of the arbitrary thresholds of the sequence method of BRS assessment in patients with heart failure and normal controls has a profound effect on the number of valid sequences obtained and on the value of BRS. Altering the blood pressure shift, whilst having no effect on the measured value of BRS, can dramatically increase the number of valid sequences. In both groups, a blood pressure shift of 0 gave the greatest number of sequences. Varying the threshold correlation coefficient of the regression lines affected the number of valid sequences, but had no effect on the measured value of BRS. Finally, we found that altering the blood pressure and R–R interval thresholds away from their standard values affects not only the measured values of BRS, but also their agreement with other BRS measurement techniques.

The clinical implications of these findings are that the optimal thresholds vary, depending on whether the individual has normal or attenuated BRS, that a single threshold may not be applicable to both groups, and that values of BRS from different centres or studies, obtained using different threshold criteria, may not be directly comparable.

**References**