Mid- and Long-term Reproducibility of Noninvasive Measurements of Spontaneous Arterial Baroreflex Sensitivity in Healthy Volunteers

Daniel Herpin and Stéphanie Ragot

Baroreflex sensitivity (BRS) is altered in a variety of circumstances and could be considered as a marker for the prognosis of some cardiovascular diseases. The present study was designed to evaluate the reproducibility of noninvasive measures of BRS, both at mid- and at long-term. Fourteen healthy volunteers were examined on three occasions (first interval = 1 week, second interval = 1 year). Each recording was performed using a noninvasive photoplethysmographic device (Finapres 2300, Ohmeda), both in supine and standing positions. Two different methods of measurement were used: the sequences method and cross spectral analysis.

The reproducibility of BRS measures was as satisfactory at mid- as at long-term for the sequences method (intraclass coefficient [ICC] = 0.87 and 0.86, respectively), but it was better at mid- than at long-term for the cross-spectral analysis (ICC = 0.85 and 0.54, respectively). The measures performed in standing position were obviously more reproducible than those made in recumbent position (ICC = 0.87 and 0.70 for the sequences method, 0.85 and 0.71 for the cross-spectral analysis, respectively). Due to the high reproducibility of these noninvasive measures, the number of patients to be included in a pharmacological study was calculated as rather small: for example, only 20 patients are required for detecting a change in upright BRS of 3 msec/mm Hg, at long-term (sequences method). Likewise, the magnitude of the regression to the mean, which has to be expected in patients selected for a follow-up study, turned out to be low: for example, <15% of the difference between the patient group mean value and the reference value, both at mid- and at long-term (standing position, sequences method). We conclude that: 1) The noninvasive measures of BRS in standing position are reproducible enough to allow longitudinal studies to be conducted over either a short or a long period; 2) The long-term reliability of the sequences method seems to be higher than that of the cross-spectral analysis; and 3) Subtle changes in SBR may be noninvasively detected within small patient groups. Am J Hypertens 1997;10:790–797 © 1997 American Journal of Hypertension, Ltd.

KEY WORDS: Baroreflex sensitivity, reproducibility, repeatability, photoplethysmography.
Baroreflex sensitivity (BRS) has been reported to be modified in numerous circumstances: elderly, systemic hypertension, heart failure, myocardial infarction, ventricular arrhythmias. Plethysmographic finger blood pressure (BP) recording has been recently used for a non-invasive determination of spontaneous cardiac BRS. The reliability of this new method has been evaluated against invasive methods; to date, however, limited information is available on its reproducibility.

Of course, the prerequisite for the use of noninvasive BRS measurement in clinical pharmacological studies is the demonstration of its repeatability over time. Therefore, the present study was designed to examine both the mid- and long-term reproducibilities of BRS noninvasive measurements in healthy volunteers. An additional objective was to use these results 1) to construct a sample size table of the number of subjects to be included in pharmacological studies; and 2) to quantify the magnitude of the regression to the mean, which has to be expected in longitudinal studies.

Methods

Subjects Data were obtained from 14 normotensive subjects: seven women and seven men, mean age 31 (±10 years [range, 23 to 51 years]), who gave their informed consent for the study. Each subject supplied a medical history and underwent a physical examination in order to exclude those with diabetes, heart disease, or acrosyndrome, and those taking medications known to interfere with BP and heart rate (HR) control.

Experimental Protocol Each subject underwent three noninvasive plethysmographic recordings (Finapres model 2300E, Ohmeda Monitoring Systems, Englewood, CT). Two recordings were performed 1 week apart, and the third was performed 1 year later. The cuff was fitted to the third finger of the right hand and located at heart level. All recordings were performed by the same operator and were started only when clinic systolic BP differed from plethysmographic measure, by <10 mm Hg, after a 10-min rest in supine position. Each recording consisted of two 15-min periods, the first in supine and the second in standing position. Both periods took place after an adaptation period of about 3 min, in order to obtain a stable situation. Data recordings were performed between 9 AM and 11 AM, with each subject examined at the same time for all of his or her recordings, in order to exclude changes related to diurnal sympathovagal variations.

BRS Estimation The analogue BP signal was sampled at 200 Hz and digitized at 12 bits by a personal computer (model 486DX2/33, Advantech PC-Labcard 718, IBM Corporation, Lexington, MA) using ISN software (Grenoble, France) to obtain values for SBP, DBP, and HR. The signal was displayed by an interactive software to detect and eliminate morphologic aberrations of BP tracing. BRS was estimated using two different methods: the sequences method and cross-spectral analysis.

The sequences method computed sequences of three or more beats in which SBP and pulse interval progressively increased (SBP+/RR+ sequences) or decreased (SBP-/RR-sequences). The threshold change was set at 1 mm Hg for SBP and at 4 msec for pulse interval. For each sequence the correlation coefficient between the two values was verified not to be <0.95. The slope was taken as a measure of the BRS, as done when changes in BP and HR are induced by intravenously administered boluses of vasoactive drugs.

The cross-spectral analysis of SBP and HR has been validated by Robbe et al. and De Boer et al. The spectral characteristics of the stationary segments of the whole recording were calculated by a direct fast-Fourier transform algorithm for discrete time series of 256 points (periodogram method). The modulus of the transfer function between SBP and RR intervals was examined in midfrequency band (66 to 127 MHz) and was considered as an index of BRS when the coefficient of coherence was >0.5.

Statistical Analysis The data obtained at the first visit (D1) were plotted against those obtained at the second visit (W1), as well as against those recorded at the third visit (Y1); the correlation coefficients were calculated by using the Spearman test, and the intervisit differences were evaluated by both a paired Wilcoxon test and an analysis of variance.

According to the recommendations of Bland and Altman, the intervisit differences (W1 − D1 and Y1 − D1) were plotted against the average values [(W1 + D1)/2 and (D1 + Y1)/2], in order to investigate any possible relationship between the measurement error and the estimated true value. The 95% confidence intervals of the differences were then calculated. In addition, the repeatability coefficients were estimated as recommended by the British Standards Institution (square root of the sum of the squares of the differences, divided by the number of subjects), allowing the 95% confidence intervals of the expected intervisit differences to be calculated.

The intraclass correlation coefficient (ρ) was calculated as follows:

\[ \rho = \frac{(BMS - WMS)}{(BMS + WMS)} \]  

where BMS and WMS denote between-subject mean squares and within-subject mean squares, respectively. Arbitrarily, a value of ρ in the range 0 to 20 indicates slight reliability; 0.21 to 0.40, fair reliability; 0.41 to 0.80, moderate reliability; and 0.81 to 1.00, strong reliability.

The number (n) of patients to be included in a study aimed at detecting a given variation in BRS, (parallel design), was calculated by using the formula:

\[ n = M \times \left( \frac{\sigma^2}{\Delta^2} \right) \]
where \( \varepsilon \) denotes the standard deviation of the intervisit differences and \( \Delta \) denotes the absolute change in BRS to be detected. \( M \) is given by the usual tables [unilateral test: \( M = 2(z_{1-\alpha} + z_{1-\beta})^2 \), bilateral test: \( M = 2(z_{1-\alpha/2} + z_{1-\beta/2})^2 \)].

Finally, the expected regression to the mean (R) was quantified according to the formula:

\[
R = (1 - r) \times (S - N) \quad \text{(formula 3)}
\]

where \( S \) denotes the BRS values of the patient group selected for a given study and \( N \), those of a reference group; \( r \) is the ratio between subject variance/total variance, as provided by an analysis of variance with repeated measures (random-effects model, nested design).\(^{22,23} \)

All analyses were performed using BMDP software (BMDP Statistical Software Inc., Los Angeles, CA) (programs 1D, 3D, 2R, 4F, 2V, and 8V). The null hypothesis was rejected when \( P > .05 \).

**RESULTS**

Table 1 shows that BRS values did not significantly differ from one examination to the other, especially in standing position, whatever the method of measurement and the interval between the two measures. The correlation coefficients between each pair of data, which measure the strength of the relation, were higher in standing position (mid-term: 0.81 to 0.84, \( P < .001 \); long-term: 0.64 to 0.81, \( P < .01 \)) than those observed in supine position (mid-term: 0.62 to 0.73, \( P < .02 \); long-term: 0.46 to 0.49, \( P = \text{NS} \)). No significant differences were found between men and women with respect to either the BRS values or the reproducibility of the measures.

According to the method of Bland and Altman,\(^ {19} \) Figures 1 to 3 plot the differences between the pairs of data against their means. It can be seen that: 1) The mean difference was close to zero (at least not significantly different from 0; thus, we were able to use the data to assess repeatability); 2) There was no obvious relation between the differences, which reflect the measurement error, and the mean, which is likely to be close to the true value; thus our data had not to be log-transformed; and 3) the degree of agreement (inversely related to the width of the 95% confidence interval of the differences) was greater in standing position than in recumbent position.

Values of the coefficients of repeatability, as well as those of the 95% confidence intervals of the expected intervisit differences, are shown on Table 2. The intraclass correlation coefficients are displayed on Figure 4. Once again, measurements performed in standing position turned out to have the highest repeatability.

**Table 1. BRS VALUES (msec/mm Hg)**

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Week 1</th>
<th>Year 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supine Position</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP+/RR+</td>
<td>21.31 ± 15.54</td>
<td>16.36 ± 10.43</td>
</tr>
<tr>
<td>SBP-/RR−</td>
<td>17.71 ± 6.44</td>
<td>16.21 ± 9.72</td>
</tr>
<tr>
<td>MF Gain</td>
<td>12.8 ± 5.0</td>
<td>11.1 ± 4.7</td>
</tr>
<tr>
<td>Standing Position</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP+/RR+</td>
<td>8.58 ± 3.73</td>
<td>8.03 ± 3.78</td>
</tr>
<tr>
<td>SBP-/RR−</td>
<td>8.43 ± 3.45</td>
<td>7.94 ± 3.72</td>
</tr>
<tr>
<td>MF Gain</td>
<td>9.7 ± 2.8</td>
<td>9.5 ± 2.5</td>
</tr>
</tbody>
</table>

All differences (Day 1 v Week 1, Day 1 v Year 1) not significant.

\( SBP+/RR+, \ SBP-/RR−, \) sequences method (see text for explanation).

MF Gain, cross-spectral analysis in mid-frequency band.

**Table 2. REPEATIBILITY COEFFICIENTS (RC) OF BRS MEASUREMENTS AND 95% CONFIDENCE INTERVAL (CI) OF THE EXPECTED ERROR (msec/mm Hg)**

<table>
<thead>
<tr>
<th></th>
<th>RC</th>
<th>95% CI of Expected Error</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mid-term (1 week)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine position</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP+/RR+</td>
<td>10.42</td>
<td>±20.42</td>
</tr>
<tr>
<td>SBP-/RR−</td>
<td>7.37</td>
<td>±14.44</td>
</tr>
<tr>
<td>MF gain</td>
<td>3.75</td>
<td>±8.17</td>
</tr>
<tr>
<td>Standing position</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP+/RR+</td>
<td>1.94</td>
<td>±4.19</td>
</tr>
<tr>
<td>SBP-/RR−</td>
<td>1.69</td>
<td>±3.65</td>
</tr>
<tr>
<td>MF gain</td>
<td>1.55</td>
<td>±3.38</td>
</tr>
<tr>
<td><strong>Long-term (1 year)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine position</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP+/RR+</td>
<td>12.01</td>
<td>±25.94</td>
</tr>
<tr>
<td>SBP-/RR−</td>
<td>6.61</td>
<td>±14.28</td>
</tr>
<tr>
<td>MF gain</td>
<td>4.66</td>
<td>±10.25</td>
</tr>
<tr>
<td>Standing position</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP+/RR+</td>
<td>2.03</td>
<td>±3.98</td>
</tr>
<tr>
<td>SBP-/RR−</td>
<td>1.84</td>
<td>±3.61</td>
</tr>
<tr>
<td>MF gain</td>
<td>2.23</td>
<td>±4.37</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.
From the preceding data, we constructed Table 3, which gives the number of subjects to be included in pharmacological studies, and Table 4, which displays the value of the 1st term of formula 3, allowing for an easy calculation of the magnitude of the regression to the mean, which has to be anticipated in follow-up studies. Investigators will only have to multiply this value by the difference (S - N).

**DISCUSSION**

The salient findings of our study are the following: 1) The measures performed in recumbent position were by far, less reproducible than those made in standing position; 2) The reproducibility of noninvasive measures of BRS was roughly the same at mid- as at long-term for the sequences method, whereas it was higher at mid- than at long-term for the cross-spectral analysis; and 3) Due to the high reproducibility of noninvasive BRS measures in standing position, very few subjects are needed for detecting a slight change in this parameter. Likewise, the magnitude of the expected regression to the mean is likely to be low.

**BRS Measures** Several invasive methods have been proposed for activating or deactivating baroreceptor reflexes: intravenous injection of vasopressor (such as...
angiotensin or phenylephrine) or vasodepressor (such as nitroglycerin) drugs, which allows the vagal control and, to a lesser extent, the sympathetic control of HR to be studied; neck chamber technique, which allows for a simultaneous investigation of BP and HR control; and low body negative pressure technique, which is the reference method for investigating the role of the cardiopulmonary receptors. More recently, computer-assisted processing of BP signals has allowed accurate measures of BRS to be noninvasively obtained from spontaneous variations of BP and HR. Of course, noninvasive BRS measures have some drawbacks, as compared with the classic phenylephrine method: the measuring period has to be longer for the noninvasive than for the invasive method (5 to 20 min, according to the different investigators, versus 1 min following intravenous injection). In addition, more computing power is required and stationary time series are needed. However, the advantages of noninvasive measures over phenylephrine method have to be emphasized: 1) No rise in BP is induced; 2) The short-term BP regulation and the BRS are not affected by the

| TABLE 4. ESTIMATION OF THE REGRESSION TO THE MEAN: VALUES OF (1-R) (SEE TEXT FOR EXPLANATION) |
|--------------------------------------------------|--------------------------------------------------|
| **Mid-Term (1 week)** | **Long-Term (1 year)** |
| SBP+/RR+ | SBP–/RR– | MF Gain | SBP+/RR+ | SBP–/RR– | MF Gain |
| Supine | 0.30 | 0.40 | 0.29 | 0.32 | 0.34 | 0.55 |
| Standing | 0.13 | 0.11 | 0.15 | 0.14 | 0.13 | 0.46 |

Abbreviations as in Table 1.
measuring method itself; 3) Measures can be applied repeatedly; and 4) Computation of modulus or sequences results in stable and high correlated values.16–18 Recently, good short-term reproducibility has been demonstrated for the sequences method, 24 h apart,14 even during the Valsalva maneuver15; likewise, cross-spectral analysis has been shown to provide reproducible results at a 1-week interval.12 Our study is the only known study comparing the reproducibilities of both methods.

The higher reproducibility of standing recordings, as compared with that of supine measures, has already been reported by Iellamo et al,14 who found a lower variation coefficient for the former than for the latter (13.9% v 15.0%), despite a lower average value (9 v 22 ms/mm Hg). We may speculate that the greater reproducibility of standing measures is mainly linked to the orthostatism-induced stimulation of sympathetic activity, resulting in a substantial decrease in BRS and, above all, in a lesser influence of the internal (or even external) stimuli on the sympathovagal balance. Eventually, intervisit variations are of lesser magnitude in standing position than in supine position, because 1) the baseline BRS level is lower, and 2) the subject is more stationary.

Clinical Implications Changes in BRS may occur with different pharmacological drugs: β-blockers,24,25 angiotensin converting enzyme inhibitors,26 centrally acting antihypertensive agents,27 α1-blockers,28 calcium antagonists,29 and digitalis.30,31 Our data clearly show that long-term reproducibility of noninvasive BRS measures is satisfactory enough, for allowing pharmacological studies to be conducted over a long period (up to 1 year), provided that: 1) the sequences method (rather than the cross-spectral analysis) is used, and 2) the recordings are performed in standing position. Of course, this assumption may be extrapolated to a shorter follow-up period of 1 to 3 months; however, a 6-month interval would probably not be adequate because of a possible seasonal rhythm in autonomic activity.32 When a pharmacological study

FIGURE 3. Relationship between average BRS values and intervisit differences in BRS measures, according to the cross spectral analysis in the mid-frequency band (66 to 127 MHz).
is designed to evaluate the mid-term effect of a drug, both methods seem to have an equivalent reliability, once again with the clear evidence that the standing position provides the most reproducible results. Furthermore, our findings should help the design of a pharmacological study to be more precisely established: slight changes in BRS may be detected within a small patient group, even at long-term, as long as the recording is made in upright position (for example, 20 patients, for detecting a drug-induced change of 3 msec/mm Hg). Finally, the quantification of the regression to the mean should allow the results of an open, noncontrolled study to be properly analyzed. It is worth noting that the magnitude of the regression toward the mean is much greater in supine than in standing position, whereas it is of the same extent at mid- as at long-term, at least for the sequences method. Interestingly, the value of the expected regression to the mean is proved to be substantially lower (−50%), than that calculated for the measure of left ventricular mass index.33

Study Limitations  By design, the standing observation period followed the supine period, raising the question of a possible “order effect.” This issue has not been specifically addressed in the literature. However, the lack of training effect with noninvasive recordings has been clearly demonstrated.13 Furthermore, Robbe et al17 emphasized that an adaptation period of about 1 min should usually be enough to obtain a stationary situation. Hence, the influence of the timing of the recordings on their reproducibility should be of limited importance, as opposed to the preeminent role of orthostatism-induced changes in autonomic activity.

Our study was conducted within a healthy population. However, the variability we found in our subject group should not be higher in diseased patients, as long as no pathological status has been found to be associated with a significant increase in baseline levels of BRS. Of course, further studies are required within selected populations, especially hypertensive patients and the elderly, to determine whether the noninvasive measurements of spontaneous BRS may prove to be valuable in clinical practice.

REFERENCES

FIGURE 4. Values of the intraclass correlation coefficient and of the corresponding reliability, according to the method of BRS measurement, the recording position, and the between-visit interval.
aging on blood pressure variability in resting conditions. Hypertension 1994;24:120–130.


