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Applicability and Clinical Relevance of the Transfer Function Method in the Assessment of Baroreflex Sensitivity in Heart Failure Patients

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OBJECTIVES We sought to assess applicability, clinical correlates, and prognostic value of the transfer function method for measuring baroreflex sensitivity (TF-BRS).

BACKGROUND Abnormalities in autonomic reflexes play an important role in the development and progression of chronic heart failure (CHF). Simple and non-invasive techniques for clinical measurement of such reflexes are desirable.

METHODS In 317 stable CHF patients in sinus rhythm (median age [interquartile range]: 54 years [48 to 59 years], New York Heart Association [NYHA] functional class II to III: 88%, left ventricular ejection fraction [LVEF]: 27% [22% to 33%]) we recorded electrocardiograms and non-invasive arterial pressure during paced breathing to measure TF-BRS.

RESULTS Owing to a high number of ectopic beats, TF-BRS could be computed in 72% of the patients; TF-BRS was lower in NYHA functional class III to IV and mitral regurgitation 2 to 3 ($p < 0.0005$ for both). Correlation with LVEF and standard deviation of all normal-to-normal intervals was 0.18 and 0.31 ($p < 0.001$ for both). During a mean follow-up of 26 months, 23% of the patients experienced a cardiac event. A depressed TF-BRS (≤3.1 ms/mm Hg) was significantly associated with the outcome (hazard ratio 3.2, 95% confidence interval [CI] 1.7 to 6.0, $p = 0.0003$). Patients with a missing TF-BRS had a high event rate (36%). Combining this information with available TF-BRS measurements, a new prognostic index could be computed in 97% of the patients that significantly predicted the outcome after adjustment for clinical and functional variables (hazard ratio 2.5, 95% CI 1.3 to 4.6 $p = 0.004$).

CONCLUSIONS In CHF patients in sinus rhythm, TF-BRS conveys relevant clinical and prognostic information, but its measurability is markedly affected by ectopic activity. Nevertheless, a TF-BRS–based risk index carrying significant and independent prognostic information can be computed in almost all patients. (J Am Coll Cardiol 2005;46:1314–21) © 2005 by the American College of Cardiology Foundation

Neurohormonal activation is a key factor in the development and progression of chronic heart failure (CHF) (1,2). Although the mechanisms accounting for this excitatory state have not been fully elucidated yet, it has long been hypothesized that abnormalities in arterial and cardiopulmonary reflexes play an important pathogenic role by reducing the restraining influence on the sympathetic nervous system and the excitatory influence on the vagal outflow to the heart (3–5). Therefore, the assessment of baroreceptor reflex function has been included within the set of relevant methods to investigate the pathophysiology of abnormal autonomic regulation in patients with heart failure (6–9).

Besides its relevance in basic research, measuring the baroreflex has also been shown to be a source of valuable information in the clinical management of CHF patients, particularly in prognostic evaluation and assessment of treatment effect (10–12), and many efforts have been devoted to the development of non-invasive methods that do not require the use of vasoactive drugs (13–15). These techniques, based on the analysis of spontaneous oscillations of arterial pressure and RR interval, are very attractive in terms of simplicity, patient acceptability, and cost.

We have focused our attention on the transfer function (TF) method for measuring the baroreceptor-heart rate reflex sensitivity (TF-BRS) (16), on the ground of its sound mathematical foundations and because it allows a clear definition of the oscillatory components that contribute to BRS measurement. Major methodological issues related to the practical use of this technique, particularly in situations of a markedly depressed reflex and/or poor signal-to-noise ratio, have recently been investigated (17), and new application criteria developed (18,19).

The present investigation was undertaken to assess the applicability and clinical relevance of the TF-BRS method in the setting of moderate-to-severe CHF patients. Particularly, we aimed at determining: 1) the actual proportion of patients in whom the measurement can be performed (i.e.,

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Abbreviations and Acronyms

AUC = area under the curve
BRS = baroreceptor-heart rate reflex sensitivity
CHF = chronic heart failure
ICD = implantable cardioverter-defibrillator
LF = low frequency
LVEF = left ventricular ejection fraction
NYHA = New York Heart Association
ROC = receiver-operating characteristic
SAP = systolic arterial pressure
SDNN = standard deviation of all normal-to-normal intervals
TF = transfer function

the measurability of TF-BRS); 2) its relationship with clinical and functional indicators of heart failure; and 3) its prognostic value. We believe that addressing these points is crucial to determine whether the TF-BRS method can be considered a potential candidate for pathophysiological investigations and clinical applications.

METHODS

Study patients. From January 1996 to August 2002, 633 consecutive patients with dilated cardiomyopathy and moderate-to-severe heart failure, were referred to the Heart Failure Unit of the Institute of Montescano for evaluation and treatment of heart failure, including evaluation for heart transplantation. One hundred sixty-two patients were excluded because of atrial fibrillation, atrial flutter, or pacemaker implantation, which did not allow analysis of cardiovascular variability. Of the remaining 471 in sinus rhythm, 122 were excluded mainly because they were clinically unstable or had had a recent (within six months) myocardial infarction or cardiac surgery. A further 32 patients were excluded owing to incomplete examinations, which did not allow performance of multivariable statistical analysis. This led to a final sample of 317 cases available for the study. All patients gave written informed consent and the study was approved by the local ethics committee.

Experimental protocol. Subjects were studied in the morning, in the supine position. The experimental protocol was carried out in our laboratory for autonomic evaluations and comprised: 1) instrumentation, patient's familiarization with paced breathing (see the following text), and signal stabilization (about 20 min); 2) 8-min recording of an electrocardiogram, lung volume (Respitrace Plus, Non-Invasive Monitoring Systems, Miami Beach, Florida), and non-invasive arterial blood pressure (Finapres 2300, Ohmeda, Louisville, Kentucky) during spontaneous breathing; and 3) 8-min recording of the same signals during paced breathing at 15 breaths/min (0.25 Hz).

To perform paced breathing, subjects were asked to follow a played-back human voice recording indicating inspiratory and expiratory phases. To improve subjects' comfort, the breathing frequency was increased or decreased upon request within ±10% of the preset value (i.e., between 13.5 and 16.5 breaths/min).

Signal preprocessing. The electrocardiographic signal was manually edited to correct for wrong QRS detections and classify ectopic beats. The RR interval (resolution 1 ms) and systolic arterial pressure (SAP) time series were then automatically calculated.

Computation of TF-BRS. The TF-BRS was computed on paced breathing recordings. The reasons for this choice are two-fold. First, the TF method requires the SAP and RR oscillations in the low frequency (LF) band (0.04 to 0.15 Hz) to be of non-respiratory origin (20,21); therefore, keeping the breathing frequency out of this band is needed (22). Second, abnormal breathing patterns are common in patients with CHF and exert a confounding effect on cardiovascular variability measurements (23,24). Paced breathing is effective in removing these patterns (23) and does not alter TF-BRS (25).

All signals (including lung volume) were plotted together on the PC screen and the widest sub-record free from artifacts, large transients, or marked changes in the fluctuation pattern of the signals interactively selected (26,27). Recordings shorter than 3 min were excluded from subsequent analysis as the reliability of TF estimates would become intolerably low (17).

Isolated ectopic beats were corrected (linear interpolation), provided that: 1) their inclusion allowed a significant increase of the analysis window; 2) they were preceded or followed by at least 3 minutes of ectopy-free recording; and 3) inclusion of the corrected beat did not change BRS by more than 15% (a threshold obtained by computer simulations). The rationale for this procedure is that ectopic beats (Fig. 1A), as well as the corrections performed on them (Fig. 1B), might heavily affect TF-BRS measurements.

The transfer function between SAP and RR interval time series was obtained from bivariate spectral analysis (weighted covariance method, 0.03 Hz-bandwidth Parzen window) (17). For each frequency, the modulus of this function, or gain, tells us how the amplitude of a sinusoidal SAP wave is transformed into the amplitude of the corresponding RR interval and is expressed in milliseconds per millimeters of mercury (ms/mm Hg). The TF-BRS was finally computed by averaging the gain function across the LF band (19).

Clinical evaluation, laboratory testing, and follow-up. Within one week from autonomic evaluation, standard clinical and laboratory examinations, including two-dimensional echocardiography, cardiopulmonary exercise testing, 24-h Holter recording, and routine blood test were performed. All measurements were entered into a dedicated database.

Time-event information during the follow-up was obtained through periodic controls in the hospital, chart review, or telephone interview of patients, relatives, or referring physician.

Statistical analysis. The association between TF-BRS and categorical variables (e.g., etiology) was assessed by the Mann-Whitney *U* test, whereas the association with con-

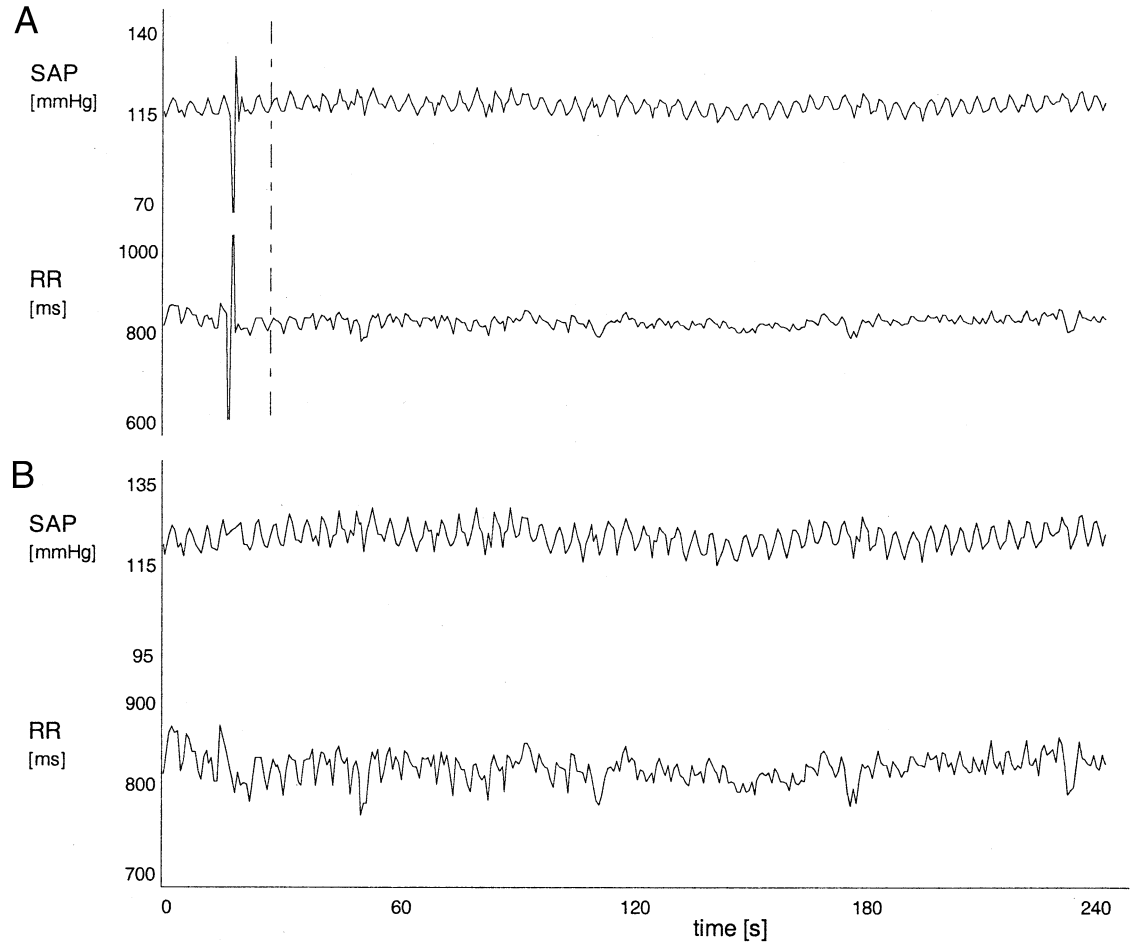

Figure 1. Representative example of the dramatic effect that a single isolated ectopic beat and its correction might have in the measurement of baroreflex sensitivity by the transfer function method (TF-BRS). Tracings **(A)** show systolic arterial pressure (SAP) and RR interval time series with a ventricular premature complex at the beginning of the recording. Measurement of TF-BRS on these signals gives 1.2 ms/mm Hg, whereas excluding the ectopic beat from the computation (i.e., starting the analysis window from the **dashed bar**) gives 6.2 ms/mm Hg. Tracings **(B)** show the same signals after correction of the ectopic beat by linear interpolation. Despite the apparent negligible effect on the fluctuation pattern of the two signals, TF-BRS becomes 4.7 ms/mm Hg, that is, −24% compared with the measurement obtained without the ectopic beat. Although the effect of the correction is not always so dramatic, this example highlights the need to perform a careful check every time a correction is made.

tinuous variables (e.g., left ventricular ejection fraction [LVEF]) was assessed by linear correlation analysis. A $p < 0.05$ was considered statistically significant and all tests were two-sided.

End point of survival analysis was total cardiac death, including appropriate and documented implantable cardioverter-defibrillator (ICD) discharge for fast ventricular tachycardia, or ventricular fibrillation and urgent transplantation (i.e., transplantation carried out upon patients requiring ventricular support or in-hospital intensive care). Patients who underwent elective heart transplantation as well as patients who died of non-cardiac causes were censored at the time of the event.

To assess the ability of TF-BRS to discriminate between patients who did and did not experience the study outcome at two years, we computed the AUC (i.e., the area under the receiver-operating characteristic [ROC] curve), after exclusion of patients with censored follow-up at <2 years. An AUC value of 0.5 indicates no predictive discrimination, whereas a value of 1 indicates perfect separation of patients with different outcomes. Two years was chosen because it was close to the mean follow-up of the patients. The AUCs were compared by the Hanley-McNeil test.

The Kaplan-Meier method was used to estimate survival curves and compute rates of survival (together with 95% confidence intervals [CIs]) at one, two, and three years. Survival curves from different subgroups of patients were compared by the log-rank test.

The association between TF-BRS and survival was assessed by the Cox proportional hazards model, after dichotomization of the measurement according to the cut point that maximized the hazard ratio. To this purpose, we calculated the hazard ratio for progressively increasing TF-BRS values comprised between the 20th and 50th percentiles (to have an adequate number of patients in each subgroup) and identified the point at which the hazard ratio attained its maximum.

Because failure to have a measurable BRS was associated

with an increased risk of event, this information was used as surrogate prognostic information in place of TF-BRS. Accordingly, a risk index (TF risk index) was obtained in all patients, regardless of having a measurable BRS.

The predictive value of the TF risk index was assessed by univariable and multivariable Cox analysis. The latter was carried out using all clinical and functional parameters with at least a weak association with the outcome ($p < 0.15$) as adjusting factors. Model-building was carried out by a stepwise procedure, with $p = 0.25$ for entering a variable into the model and $p = 0.15$ to remain into the model. At the end of the stepwise process, only variables with $p < 0.05$ were retained in the final multivariable model. The assumption of proportional hazards was assessed by plotting Schoenfeld residuals.

Owing to the marked skewness in the distribution of some variables (including TF-BRS), descriptive statistics are given as median, lower, and upper quartile, unless otherwise stated. The rate of ectopic beats is expressed as events per hour both for long-term and short-term recordings. All analyses were performed with the SAS/STAT statistical package, release 8.02 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Demographic and clinical characteristics of the study patients are given in Table 1.

Measurability of TF-BRS. Of the 317 recordings carried out for the computation of TF-BRS, 81 (26%) could not be analyzed, owing to a high number of ectopic events (113 [75 to 234]/h). Most of the events were of the ventricular type (95 [58 to 200]/h). Correlation with Holter arrhythmia data was moderate both for ventricular ($r = 0.48$, $p < 0.0001$) and supra-ventricular beats ($r = 0.44$, $p < 0.0001$). Six recordings were excluded due to artifacts or large signal transients, whereas another two were excluded due to improper execution of paced breathing. Isolated ectopic beats were corrected in 45 recordings. Thus, the analysis of TF-BRS was carried out in 228 recordings, that is, 72% of those available for the study. The median duration of analyzed recordings was 287 s (range 180 to 491 s), with a median TF-BRS of 3.5 (1.7 to 6.6) ms/mm Hg. Mean coherence across the LF band was 0.29 (0.19 to 0.41).

Clinical correlates of TF-BRS. As shown in Table 2, TF-BRS was slightly lower in patients with ischemic compared with idiopathic cardiomyopathy, but the difference did not reach statistical significance ($p = 0.17$). A significant decrease of BRS was found in patients with New York Heart Association (NYHA) functional class III to IV and in those with mitral regurgitation grade 2 to 3. We did not find any association between TF-BRS and non-sustained ventricular tachycardia ($p = 0.44$). Low values of the correlation coefficient were found between TF-BRS and both LVEF ($r = 0.18$) and standard deviation of all normal-to-normal intervals (SDNN) ($r = 0.31$), yet the association was highly statistically significant ($p = 0.009$ and $p < 0.0001$ respectively).

Table 1. Demographic, Clinical, and Functional Characteristics of the Study Sample (n = 317)

Characteristic	
Age (yrs)	54 (48–59)
Gender (% male)	84
NYHA functional class (%)	
I	10
II	51
III	37
IV	2
Cause of CHF (%)	
Ischemic cardiomyopathy	48
Idiopathic cardiomyopathy	40
Other	12
Resting systolic arterial pressure (mm Hg)	110 (100–125)
Resting diastolic arterial pressure (mm Hg)	70 (70–80)
LVEF (%)	27 (22–33)
LVESD (mm)	59 (50–66)
LVEDD (mm)	70 (63–77)
Mitral regurgitation 2 to 3 (%)	32
Peak V_{O_2} (ml/kg/min)	15 (12–18)
24-h mean RR interval (ms)	800 (722–897)
VPCs (No./h)	16 (3–71)
NSVT (%)	39
SDNN (ms)	89 (63–118)
BUN (mg/dl)	48 (39–57)
Sodium (mEq/l)	140 (138–142)
Creatinine (mg/dl)	1.13 (0.99–1.31)
Potassium (mEq/l)	4.4 (4.1–4.6)
Bilirubin (mg/dl)	0.74 (0.53–1.01)
Medical therapy (%)	
ACE inhibitors	85
Diuretics	84
Nitrates	49
Beta-blockers	42
Amiodarone	22

Continuous variables are expressed as median (lower quartile to upper quartile).

ACE = angiotensin-converting enzyme; BUN = blood urea nitrogen; CHF = chronic heart failure; LVEF = left ventricular ejection fraction; LVESD, LVEDD = left ventricular end systolic and diastolic diameter; NSVT = non-sustained ventricular tachycardia; NYHA = New York Heart Association; Peak V_{O_2} = oxygen uptake at the maximum level of exercise; SDNN = standard deviation of normal-to-normal RR intervals; VPCs/h = ventricular premature contractions.

Prognostic value of TF-BRS. During a mean follow-up of 26 months (minimum to maximum: 0.2 to 36 months), 73 (23%) of the 317 patients enrolled in the study experienced a cardiac event, including 6 urgent transplantations and 8 appropriate ICD discharges.

In the dataset of 228 patients with a measurable TF-BRS (mean follow-up: 27 months [minimum to maximum: 1.2 to 36 months]; event rate: 19%), TF-BRS was significantly lower in the patients with a poor outcome (2.2 [1.0 to 5.7] vs. 3.8 [1.9 to 6.6] ms/mm Hg, $p = 0.014$), and the AUC ± SE for a two-year outcome event was 0.68 ± 0.06. The AUC for the continuous variables shown in Table 1 ranged from 0.54 ± 0.06 for 24-h mean RR ($p = 0.025$ vs. TF-BRS) to 0.66 ± 0.05 for SAP ($p = 0.40$ vs. TF-BRS). A graphical example is given in Figure 2, where we have superimposed the ROC curves of TF-BRS and LVEF; in

Table 2. Association Between TF-BRS and Major Clinical Variables With Categorical Scale (n = 228)

	TF-BRS (ms/mm Hg)
Etiology	
Ischemic cardiomyopathy	3.3 (1.5–5.9)
Idiopathic cardiomyopathy	4.0 (2.0–7.1)
NYHA functional class	
I–II	4.3 (2.4–8.0)
III–IV	2.2 (1.1–4.2)*
Mitral regurgitation	
0–1	4.1 (2.3–7.4)
2–3	2.2 (1.1–5.2)†
NSVT	
No	3.5 (1.8–6.6)
Yes	3.3 (1.6–6.1)

*p < 0.0001 vs. NYHA class I–II; †p = 0.0001 vs. mitral regurgitation 0–1.
NSVT = non-sustained ventricular tachycardia; NYHA = New York Heart Association; TF-BRS = baroreflex sensitivity by the transfer function method.

the overall region of best trade-off between sensitivity and false positive rate (1-Specificity), TF-BRS shows a superior predictive discrimination compared with LVEF.

The optimum cutoff value for dichotomizing TF-BRS was 3.1 ms/mm Hg. Kaplan-Meier survival curves are shown in Figure 3. Results from Cox analysis and standard figures of predictive accuracy for a two-year outcome event are given in the middle column of Table 3. Patients with depressed BRS showed a 3.2 increased risk of experiencing a study outcome during the follow-up, compared with those with a more preserved BRS. We did not find any evidence of an interaction effect between dichotomized TF-BRS and beta-blocker treatment (p = 0.30).

Relationship between missing measurements and survival. In the 81 patients in whom TF-BRS could not be measured because of a high number of ectopic beats, the event rate was much higher than in the subjects with a measurable BRS (36% vs. 19%, p = 0.002). In the patients in whom the measurement could not be performed for other causes, the event rate was 17%. Accordingly, a new prognostic index (TF risk index) was derived in the following manner: a patient was considered at high risk if TF-BRS was depressed (i.e., ≤3.1 ms/mm Hg) or if failure to measure TF-BRS was due to high ectopic activity; conversely, he/she was considered at low risk if TF-BRS was more preserved (i.e., >3.1 ms/mm Hg). Survival analysis was then carried out on this new dataset comprising 309 subjects (97% of the original sample).

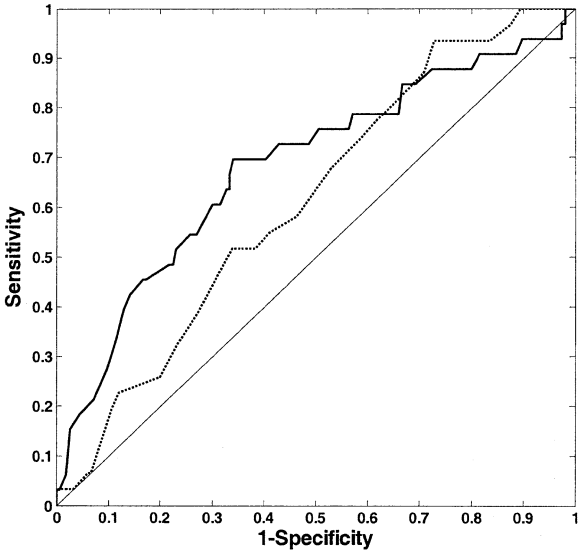

Figure 2. Receiver-operating characteristic curves for the prediction of a two-year outcome according to baroreflex sensitivity by the transfer function method (TF-BRS) **(solid line)** and left ventricular ejection fraction (LVEF) **(dotted line)**. The identity line indicates no predictive discrimination. The area under the curve was 0.68 ± 0.06 for TF-BRS and 0.61 ± 0.05 for LVEF.

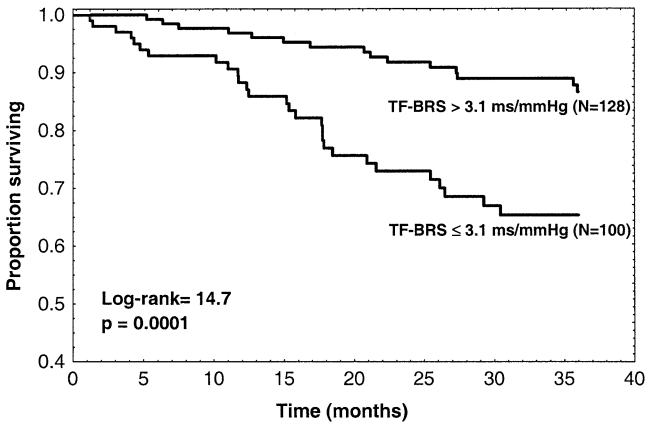

Figure 3. Kaplan-Meier survival curves according to dichotomized of baroreflex sensitivity by the transfer function method (TF-BRS) (n = 228). Estimated survival probabilities (95% confidence interval) at 12, 24, and 36 months are: 0.97 (0.94 to 1.00), 0.92 (0.87 to 0.97), 0.87 (0.80 to 0.93), respectively, for TF-BRS >3.1 ms/mm Hg; and 0.88 (0.82 to 0.95), 0.73 (0.63 to 0.82), 0.65 (0.55 to 0.76), respectively, for TF-BRS ≤3.1 ms/mm Hg.

Predictive value of the TF risk index. Univariable Cox analysis results and figures of predictive accuracy for the TF risk index are given in the far-right column of Table 3. Corresponding Kaplan-Meier survival curves are shown in Figure 4. The prognostic performance of the TF index was quite similar to that found for TF-BRS, except that sensitivity increased at the expense of specificity.

Among the clinical and functional parameters listed in Table 1, gender, NYHA functional class, resting SAP,

Table 3. Results From Cox Analysis and Figures of Predictive Accuracy for Dichotomized TF-BRS (≤3.1 ms/mm Hg vs. >3.1 ms/mm Hg) and for the TF Index

	TF-BRS (n = 228)	TF Risk Index (n = 309)
Wald chi-square	13.1	18.7
p value	0.0003	<0.0001
Hazard ratio	3.2 (1.7–6.0)*	3.5 (2.0–6.2)
Sensitivity†	70%	82%
Specificity†	67%	52%
PPV†	31%	32%
NPV†	91%	91%

*Values within brackets are 95% confidence intervals; †Computations are for a two-year outcome event.
NPV = negative predictive value; PPV = positive predictive value; TF-BRS = baroreflex sensitivity by the transfer function method.

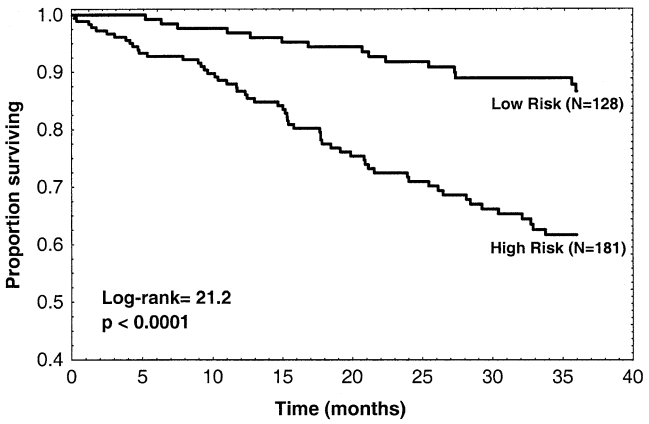

Figure 4. Kaplan-Meier survival curves according to the transfer function (TF)-index (n = 309). Subjects at high risk are those with depressed baroreflex sensitivity (BRS) (i.e., TF-BRS ≤3.1 ms/mm Hg) or having a missing BRS due to high ectopic activity, whereas subjects at low risk are those with more preserved BRS (i.e., TF-BRS >3.1 ms/mm Hg). Estimated survival probabilities (95% confidence interval) at 12, 24, and 36 months are: 0.97 (0.94 to 1.00), 0.92 (0.87 to 0.97), and 0.87 (0.80 to 0.93), respectively, for low risk; and 0.87 (0.82 to 0.92), 0.71 (0.64 to 0.78), 0.62 (0.54 to 0.70), respectively, for high risk.

LVEF, mitral regurgitation, left ventricular end diastolic and systolic dimensions, sodium, potassium, bilirubin, creatinine, blood urea nitrogen, and number of ventricular premature contractions (VPC)/h showed a p value <0.15 in univariable proportional hazards analysis. With these variables as adjusting factors in a multivariable Cox model, the TF risk index maintained a highly significant association with the outcome, with an adjusted hazard ratio of 2.5 (95% CI: 1.3 to 4.6, p = 0.004). Significant covariates remaining in the model were: mean SAP (p = 0.046), bilirubin (p = 0.006), potassium (p = 0.0006), and number of VPCs/h (p < 0.0001). There was no interaction between TF risk index and beta-blocker treatment (p = 0.32).

DISCUSSION

Simple, non-invasive, and requiring limited resources, the TF method has gained the attention of many investigators, including our group, as an ideal tool to measure the baroreceptor-heart rate reflex in the clinical setting of patients with deranged autonomic cardiovascular control. Therefore, to verify this reasonable expectation, in this study we assessed the clinical applicability and relevance of the method in moderate-to-severe CHF patients. We found that: 1) the measurability of TF-BRS was markedly affected by the high prevalence of severe ectopic activity in the studied population; 2) clinical correlates were similar to those previously found measuring BRS by the classical phenylephrine test (10); 3) a depressed TF-BRS was associated with a worse prognosis; and 4) combining the prognostic information derived from measured BRS with that related to the reasons for failed measurement, a new risk index could be obtained in almost all studied patients, carrying predictive information independent of that of most common clinical and functional indicators.

Measurability of the TF-BRS method. In its original formulation (16), the TF method required the coherence between SAP and RR interval to be ≥0.5 to guarantee reliable BRS estimates. Applying this criterion to post-myocardial infarction and CHF patients, BRS can not be measured in a considerable number of cases (19). It is easy to show that low or very low coherence values represent a "natural" phenomenon in pathological subjects, because they reflect an impaired reflex function and/or a poor signal-to-noise ratio (17,19). Moreover, the reliability of TF estimates depends on other factors in addition to the coherence (17). Therefore, new analysis criteria not based on the coherence function have been devised and evaluated (18). These theoretical and experimental results constituted the basis of the methodological approach followed in the present study.

Although, in the computation of TF-BRS, we did not use any coherence constraint, 28% of the recordings could not be analyzed, mostly owing to high ectopic activity. Taking into account that 26% of the patients admitted to our Heart Failure Unit had atrial fibrillation, atrial flutter, or an implanted pacemaker, which is another exclusion criterion for BRS estimation, the actual rate of measurability of TF-BRS in our population is near 50%.

Clinical correlates. The TF-BRS was significantly lower in patients with more severe symptoms and higher mitral regurgitation, whereas a non-significant change was observed between patients with ischemic and idiopathic cardiomyopathy and between patients with and without non-sustained ventricular tachycardia. A poor, albeit statistically significant, correlation was found with both LVEF and SDNN. These results are very similar to those previously found with the classical phenylephrine test to estimate BRS (10) and clearly suggest that, although the agreement between pharmacological and spectral measurements is poor (28,19), both techniques reflect the clinical and functional status of the patients in the same manner.

Prognostic value. A reduced TF-BRS was significantly associated with a poor outcome. This finding confirms our previous investigation in a cohort of CHF patients enrolled during the four years preceding the beginning of the present study (10) and strongly supports the notion that an impaired baroreflex plays an important role in the progression of the disease and event occurrence, despite marked changes in pharmacological treatment. Although the two-year predictive discrimination of TF-BRS, as quantified by the area under the ROC curve, was moderate (0.68), it was higher than or superimposable to the other continuous variables considered in the study.

An interesting finding of this study was the strong association between failure in BRS measurement due to high ectopic activity and the outcome, the event rate being almost double in non-measurable cases compared with the rest of the patients. Since the former constituted a remarkable portion of the study population, this result deserves further investigations to fully assess its clinical relevance. Moreover, a moderate correlation was found between the

ectopy rate measured during the paced breathing protocol and that observed in Holter recordings. Although this is likely to be due, to a great extent, to the marked differences between the two recording conditions (24-h versus short-term), we can not exclude a specific role of the paced breathing protocol. Indeed, we found that in more than 20% of the patients with failed measurement, ectopic activity was absent or markedly reduced during spontaneous breathing. This might be related to the condition of slight mental effort and mild hyperventilation that occurs during voluntary control of breathing in some patients (25).

A new risk index was derived, grouping together the patients with missing TF-BRS due to ectopic beats with those with depressed BRS. In this way, risk classification could be extended to 97% of the studied sample. This strategy proved to be successful, because not only the risk index was strongly associated with the outcome but its predictive value was substantially maintained after adjustment for all significant clinical and functional parameters.

It is noteworthy that we found the number of VPCs/h among the adjusting factors that entered the final multivariable model, although the risk index already contained some information related to ectopy rate. This seems to further support the notion that the appearance of a high number of ectopic beats (mainly ventricular) during a short-term paced-breathing recording carries a specific clinical and, perhaps, physiopathological information additive to that achievable through Holter recording.

Assumptions and limitations of the transfer function method. A few final remarks have to be made on some intrinsic methodological limitations of the TF-BRS method. As with all other measurement techniques, this method is based on a set of assumptions that represent a great simplification of the underlying physiological mechanisms. In particular, the computation of the TF between SAP and RR interval assumes an open-loop and linear link between the two signals and the absence of other influences on the RR interval in addition to the baroreflex and uncorrelated disturbances (17). Although some experimental studies provide indirect support to most of these assumptions (20,29), it is not yet known how robust the technique is in pathological subjects. Moreover, being restricted to the analysis of oscillatory components between 0.04 and 0.15 Hz, the TF method takes into account only a portion of the overall cardiovascular dynamics, whose relevance in everyday life has not yet been fully elucidated. Hence, it would be sensible to state that TF-BRS measurements "reflect" the transfer properties of the baroreflex, rather than being the "true BRS" of a given subject. Despite these limitations, however, it is worth emphasizing that the TF method does not require a pharmacological intervention to raise or lower blood pressure and is not time-consuming in terms of data acquisition.

Conclusions. Despite its theoretical and practical appeal, the measurability of baroreceptor-heart rate reflex in CHF patients by the TF-BRS method is markedly affected by the presence of severe ectopic activity. This limitation would be particularly relevant in pathophysiological investigations, because it might cause an important selection bias in the interpretation of results. In the clinical setting, however, where the identification of robust prognostic markers for CHF is of major concern, we can use the information on baroreflex sensitivity (from subjects with a measurable TF-BRS) in combination with the information on the reasons for failed measurement (from subjects with a non-measurable TF-BRS) to obtain a risk index. This index can be computed in almost all patients in sinus rhythm and provides significant and independent prognostic information.

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