Heart Failure Patients and reproducibility of the Heart Rate Turbulence Indexes

Graziamaria Corbi, Gianni D'addio, Nicola Ferrara and Mario Cesarelli

***Abstract*—** Although cardiac automaticity is intrinsic to various pacemaker tissues, heart rate and rhythm are largely under the control of the autonomic nervous system. Cardiovascular oscillations following spontaneous ventricular premature complexes (VPC) are characterized by a short-term heart rate fluctuation known as heart rate turbulence described by the so-called turbulence onset (TO) and slope (TS). Despite a recent written consensus on the standard of heart rate turbulence measurement, reproducibility data are lacking. Aim of the paper was a reproducibility study of heart rate turbulence indexes in heart failure patients. Eleven heart failure patients underwent two 24h ECG Holter recordings, spaced 7±5 days. A paired t test was used to assess the clinical stability of patients during the study period and the number of PVC in Holter recordings' couples. Both TO and TS indexes were calculated for each isolated VPC, and due to their skewed distribution, reproducibility of median and mean TO and TS was studied by Bland-Altman technique. Results showed that median heart rate turbulence indexes might be preferred to commonly suggested mean values and that, although TO showed lower bias value than TS, TS can be considered much more reproducible than TO, comparing limits of agreements with normal values. This preliminary results suggest the use of medians instead of mean heart rate turbulence indexes values and a reliability of the turbulence slope greater than the turbulence onset index.

***Keywords*—** Turbulence, Heart failure, heart rate variability

I. Introduction

Although cardiac automaticity is intrinsic to various pacemaker tissues, heart rate and rhythm are largely under the control of the autonomic nervous system.

Abnormalities of the autonomic nervous system are recognized to be part of the syndrome of heart failure.

Despite recent advances in the clinical, diagnostic tools, and therapy of Heart Failure, the incidence and prevalence of this syndrome show a steady increase [1].

G. Corbi, was with Dpt of Medicine and Health Sciences of University of Molise, Campobasso, 86100 Italy (corresponding author; phone: +39-0874-404776; fax: +39-0874-404778; e-mail: graziamaria.corbi@ unimol.it).

G. D'addio, was with S. Maugeri Foundation, IRCCS, Rehabilitation Institute of Telese-Campoli, 82037 Telese Terme, Italy (e-mail: gdaddio@fsm.it)

N. Ferrara, was with Dpt of Medical Translational Sciences, Federico II University of Naples, 80131 Naples, Italy.

M. Cesarelli, was with Dpt of Biomedical, Electronic and Telecommunication Engineering, University "Federico II", 80131 Naples (e-mail: cesarell@unina.it)

In 2009, 1 in 9 death certificates in the United States mentioned heart failure.

The number of any-mention deaths attributable to heart failure was approximately as high in 1995 as it was in 2009. Additionally, hospital discharges for heart failure remained essentially unchanged from 2000 to 2010 [2].

The progressive aging of the population, the decline of mortality in the acute phase of many cardiovascular diseases (CVD) and prolonged survival of patients with HF contribute to determine this epidemiological reality.

Based on population projections, it is estimated that over the next 30 years the incidence of HF will quadruplicate and that 50% of patients will be over eighty [1].

At 40 years of age, the lifetime risk of developing HF for both men and women is 1 in 5, and it also remains at 20% for 80 years old men and women.

Whereas survival after HF diagnosis has improved over time [3] the death rate remains high, with a mortality of about 50% within 5 years in people with diagnosed HF [4,5].

In the elderly, data from Kaiser Permanente indicate that survival after the onset of HF has also improved [6].

Among Medicare beneficiaries, the overall 1-year mortality rate declined slightly over the past decade but remains high [7].

Activation of the sympathetic nervous system, decreased activity of the parasympathetic nervous system, and depressed baroreceptor function are early features that may precede the onset of clinically obvious symptoms and signs of heart failure [8–11].

Furthermore, these abnormalities of the autonomic nervous system and neuroendocrine activation in general have an important impact on the progression and prognosis in patients with heart failure.

In fact heart failure is characterized by neurohumoral activation with sympathetic overdrive and progressive hemodynamic deterioration, with a significantly reduced heart rate variability and impaired baroreflex sensitivity [12], and these changes constitute the main feature of heart failure independently of its etiology [13]. Even though diagnosis of heart failure is based on symptoms with supporting evidence from imaging techniques, the electrocardiographic abnormalities significantly contribute to overall clinical picture and clinical decisions.

Continuous ambulatory Holter ECG monitoring is not considered a basic diagnostic method in the diagnosis of heart failure, whereas it has widely demonstrated to be a valuable tool in risk stratification.

Recently it has been demonstrated an increased interest in evaluation of dynamic Holter-derived ECG markers reflecting changes in heart rate and ventricular repolarization behavior [14].

Since changes in the sympathetic and vagal traffic to the sinoatrial node alter the natural frequency of the cardiac pacemaker inducing a corresponding change in heart rate, the measurement of the latter would be the simplest way of appraising the autonomic control of the heart and, more specifically, the sympatho-vagal balance.

The interaction between heart rate, the intrinsic frequency of the pacemaker and the levels of vagal and sympathetic outflows to the heart has been model as a multiplicative relationship. Hence, given the intrinsic frequency, the net effect of the sympatho-vagal balance at any instance is expressed by the current heart rate. However, many ECGs variations has been the subject of numerous clinical studies investigating a wide spectrum of cardiological and non-cardiological diseases and clinical conditions, that can be modified by several factors [15].

Unfortunately, however, the intrinsic frequency changes between individuals and its measurement requires a complete autonomic blockade. As a consequence, the measurement of heart rate provides only an uncalibrated quantification of the sympatho-vagal balance.

Beat-to-beat spontaneous fluctuations of heart rate do occur continuously in every human subject with an intact heart and reflect corresponding fluctuations in neural traffic of efferent vagal and sympathetic nerves. The fluctuation of heart rate around its mean has commonly been referred to as heart rate variability (HRV).

In fact the analysis of heart rate variability is a well recognized non-invasive tool to investigate the cardiovascular autonomic control especially in HF patients [16-18], but also in other clinical conditions [19-23].

Between different methods to evaluate the HRV [24,25] an indirect and noninvasive baroreflex sensitivity assessment is represented by heart rate turbulence technique, consisting on the study of short-term sinus cycle length fluctuations following spontaneous isolated ventricular premature complexes (VPCs) [26].

In recent years many methods have been used to facilitate the identification of patients at risk especially after myocardial infarction or heart failure. However, even the two most commonly used methods, such as the analysis of heart rate variability and the study of baroreflex sensitivity, have often shown difficulties in application that have limited their use.

For these and other reasons, often the risk stratification arrhythmic and the consequent choice of therapy are based on the evaluation of a single parameter such as the left ventricular ejection fraction, that, if on the one hand is able to identify patients at high mortality, on the other hand it is not so effective in identifying patients at high risk of sudden cardiac death.

In this regard, among the most innovative methods that have been developed to identify patients at risk, has recently emerged attention of researchers studying the heart rate turbulence, a method which is based on the analysis of the fluctuations of the cardiac cycle after a ventricular premature beat.

Heart rate turbulence has been described recently as an independent predictor of mortality in patients with chronic heart failure [27-33].

The physiologic pattern of heart rate turbulence consists of a brief heart rate acceleration followed by a more gradual heart rate deceleration before the rate returns to a pre-ectopic level. Available physiologic investigations confirm that the initial heart rate acceleration is triggered by transient vagal inhibition in response to missed baroreflex afferent input caused by hemodynamically inefficient ventricular contraction. A sympathetically mediated overshoot of arterial pressure is responsible for the subsequent heart rate deceleration through vagal recruitment. Hence, the heart rate turbulence pattern is blunted in patients with reduced baroreflex [34].

In the last years several trials confirm a growing interest in this technique, reporting heart rate turbulence indexes strongly correlated with the severity of heart failure [28,35] and as powerful independent predictor of decompensation and sudden death in this pathology [29,30,34].

Despite a recent written consensus on the standard of heart rate turbulence measurement and clinical use has been reached [36], a number of issues still remain poorly understood and need further investigation [37]. Particularly reproducibility data are lacking and would be of clear interest [35].

Aim of the paper was the evaluation of short-term reproducibility of heart rate turbulence indexes in patients with stabilized heart failure. Reproducibility represents a basic methodological study for clinical settings and this is the first paper addressing such issue.

II. Study Population

Eleven patients (62 ± 10 years old, males) with clinically stable heart failure in New York Heart Association functional class II-III, in sinus rhythm, admitted to the “Heart Failure Unit of “Maugeri Foundation Institute of Telese Terme" were studied.

All patients underwent two 24 hours ECG Holter recordings spaced 7±5 days.

The day after the Holter recording, were performed clinical and laboratory examinations, including 2D echocardiography for left ventricular ejection fraction (LVEF) evaluation, ECG stress test for maximal oxygen consumption (VO2max) estimation and a blood sample for plasma norepinephrine assay (NPE), assessed by a single-isotope radioenzymatic method, in all selected subjects.

III. Holter Analysis

The study population underwent to a 24-hour Holter ECG recording by a portable three-channel tape recorder, processed by a Marquette 8000 T system with a sampling frequency of 128 Hz.

All recordings were performed at admission: after the preparation of the skin, self-adhesive electrodes were placed in the positions usually used for three-leads Holter monitoring, and recording was started between 9.00 and 9.30 AM.

During the recording period the patients were allowed to standing or sitting next to their beds, while other activities were not allowed.

In order to be considered eligible for the study, each recording had to have at least 12 hours of analyzable RR intervals in sinus rhythm. Moreover, this period had to include at least half of the nighttime (from 00:00 AM trough to 5:00 AM) and half of the daytime (from 7:30 AM trough to 11:30 PM) analyzable periods [38].

Each beat was labeled as normal, VPCs or aberrant according to recognition by the algorithm for tape analysis and after an investigator's verification.

IV. Heart Rate Turbulence Analysis

By the term heart rate turbulence indicates a method that quantifies the oscillations of the cardiac cycle following the compensatory pause induced by a ventricular premature beat.

The sinus cardiac cycles that follow the compensatory pause associated with a ventricular premature beat have a biphasic framework characterized by an initial acceleration followed by a deceleration, such as to return the duration of the cardiac cycle to the one immediately preceding the ventricular extrasystoles.

The initial acceleration takes the name of turbulence onset (TO) and is quantified by the relative ratio between the duration of the two cardiac cycles (expressed in msec and measured on RR intervals) immediately preceding and the two cardiac cycles immediately following the compensatory pause, multiplied by 100. The parameter is measured on cardiac cycles preceding and following each ventricular premature beat and expressed as the mean value for each individual patient. Values of TO> 0% correspond to a deceleration after a ventricular premature beat, while values <0% corresponds to an acceleration.

With the term of turbulence slope (TS) indicates instead a measure of the slope of the linear regression of RR intervals after the compensatory pause. The value of TS is calculated on the steepest slope of a sequence of five cardiac cycles comprised between 20 RR intervals that follow the compensatory pause induced ventricular extrasystole.

In a normal subject a ventricular premature beat induces a biphasic response in the duration of sinus cardiac cycles following the compensatory pause: first acceleration is observed followed by a gradual lengthening of RR intervals up to values similar to those observed before the premature beat ventricular.

Accordingly to standards of measurements [22], heart rate turbulence was estimated by the two numerical descriptors: turbulence onset (TO), reflecting the initial phase of sinus rhythm acceleration and turbulence slope (TS), describing deceleration phase. With regard to the clinical application of this method, it was proposed to use as the threshold values for the parameter discriminating 0% TO and> 2.5 msec / RR for the parameter TS.

TO was defined as a percentage difference between the mean of the first two RR intervals following the compensatory pause after a VPCs and the last two sinus RR intervals before a VPCs.

TS was described as the maximum positive slope of a regression line assessed over any of 5 consecutive RR intervals within the first 15 sinus RR intervals after a VPCs.

Both indexes were separately calculated for each isolated VPCs.

Filtering algorithms were used to eliminate inappropriate RR intervals and VPCs with overly long coupling intervals or overly short compensatory pauses.

Filtering algorithms excluded from the heart rate turbulence calculation RR intervals with the following characteristics: <300 ms, >2000 ms, >200 ms difference to the preceding sinus interval, and >20% difference to the reference interval (mean of the 5 last sinus intervals) [36].

In addition, heart rate turbulence calculation has been limited to VPCs with a minimum prematurity of 20% and a post-extrasystole interval that is at least 20% longer than the reference interval (mean of last 5 sinus RR intervals) [36].

All analyses were performed by HRT-LAB, a customized Matlab [38] software toolbox developed by the authors.

V. Statistical Analysis

A paired t test has been used to assess the clinical stability of patients during the study period and the number of PVCs in Holter recordings' couples. Since estimation of heart rate turbulence indexes is sensitive to the number of VPC [40], effectiveness of pairing between number of studied VPC for each patient in the two studies was assessed by calculating the Pearson correlation coefficient, r, and a corresponding P value.

The variables describing the hemodynamic status, neurohormonal activation and exercise performance were analyzed by repeated measures ANOVA.

To exclude clear outliers values, the first and last percentile of the TO and TS distributions has been cut. D'Agostino-Pearson normality test ($p<0.05$) was used to assess the normality of all TO and TS distributions.

Reproducibility has been studied by Bland-Altman technique [41,42], plotting the difference between the two repeated measurements against their mean value, which can be assumed as the best estimate that we have of the true value.

Removing the variation between subjects, Bland-Altman plots describe the bias and the standard deviation respectively

as the average and the standard deviation of the difference between the two measures. The last one was used to calculate the limits of agreement, computed as the mean bias plus or minus 1.96 times its standard deviation, accordingly to definition of repeatability coefficient [43].

Any future measure should lie within the limits of agreement approximately the 95% of the time. All statistics were performed by GraphPad Software [44].

VI. Results

The study population did not show any change in clinical conditions.

The variables describing the hemodynamic status, neurohormonal activation and exercise performance, as reported in Table 1, showed relatively clinical stable conditions of the patients during the study period.

Since the heart rate turbulence indexes evaluation is based on the number of isolated VPCs detected in the Holter recording, for each patient only couples of recordings with approximately the same number of VPCs and at least 50 VPCs per Holter (Table 2) were compared.

Table 1: Assessment of patients' clinical stability during the study period

	Study 1	Study 2	p
LVEF	28 ± 7	27 ± 4	0.87
NPE	344 ± 156	351 ± 160	0.36
V02max	12 ± 2	13 ± 2	0.23

P value for all variables in third column; Left ventricular ejection fraction [%] in the first row; plasma norepinephrine levels [pg/L] in the second row; maximal oxygen consumption [(mLxkg-1x min-1] in the third row. All data expressed as mean ± standard deviation.

Table 2: Number of isolated VPCS in the Holter recordings

Study 1	Study 2	p	pairing r	pairing p
205 ± 190	154 ± 142	0.10	0.88	0.0002

P value in third column; Pearson correlation coefficient between studies and related p value in fourth and fifth column. All dana expressed as mean ± standard deviation.

Since TO and TS showed highly skewed non-gaussian distribution (D'Agostino-Pearson normality p test >0.05 for almost all recordings) their medians differed from mean values. The average median and mean TO and TS values in the two studied recordings are shown in Table 3. All bias and limits of agreement values are reported in Table 4.

Table 3: Average median and mean to and ts values in the two studies

	TO		TS	
	Study 1	Study 2	Study 1	Study 2
Median	−0.55±0.87	−0.65±1.12	6.75±3.59	7.42±3.62
Mean	−0.38±0.93	−0.60±1.46	7.44±3.95	8.57±4.55

Mean ± standard deviation values for all variables in the two studies. P value > 0.05 for all.

Table 4: Bias and limits of agreement values of Bland-Altman plots

	TO		TS	
	Median	*Mean*	*Median*	*Mean*
Bias	0.10	0.22	−0.68	−1.12
SD of Bias	0.75	1.35	1.45	1.62
95% Limits of Agreement				
From	−1.37	−2.43	−3.52	−4.30
To	1.57	2.87	2.17	2.06

In the first two rows the bias and its standard deviation between the two measures for all medians and mean TO and TS values. In the last two rows the related 95% limits of agreement

Fig. 1 and 2 show Bland-Altman plots of TO median and mean values, and Fig. 3 and 4 of TS median and mean values.

VII. Discussion

Several time- and frequency-domain indexes have been extracted from the HRV signal using digital signal processing techniques, and experimental evidence has been provided that known changes in sympathetic and vagal outflows to the heart associated with physiological maneuvers, drug administration, disease or increased risk for lethal arrhythmias are accompanied by well-defined changes in HRV parameter. It has been thus hypothesized that spontaneous cardiovascular fluctuations can be exploited to provide quantitative indexes of cardiac autonomic control mechanisms.

More recently, several investigators have shown that heart rate fluctuations share some basic properties of processes with nonlinear dynamics and chaotic determinism.

Nonlinear phenomena involved in the genesis of HRV are thought to be determined by the complex interaction of

hemodynamic, electrophysiological and humoral variables as well as by reflex and central regulatory mechanisms involved in cardiovascular regulation. It has been speculated that analysis of HRV based on methods of nonlinear dynamics may provide valuable information for the physiological interpretation of HRV and for prognostic stratification of cardiac disease patients.

However, even the two most commonly used methods, such as the analysis of heart rate variability and the study of baroreflex sensitivity, have often shown application difficulties which have limited their use.

For these and other reasons, often arrhythmic risk stratification and the consequent choice of treatment is based on the evaluation of a single parameter such as the left ventricular ejection fraction, which, if is able to identify patients at high mortality on the other hand it is so effective in identifying patients at high risk of sudden cardiac death.

In this regard, among the most innovative methods that have been developed to identify patients at risk, has recently emerged attention of researchers studying the heart rate turbulence (HRT), a method that is based on the analysis of the fluctuations of the cardiac cycle after a ventricular premature beat.

It is widely accepted that HRT is an expression of autonomic control of the sinus node. Indeed, the biphasic response is easily appreciated with particular entity in young subjects and in the night hours which, as known, are characterized by a prevailing vagal modulation, while it is markedly reduced in patients with autonomic dysautonomia.

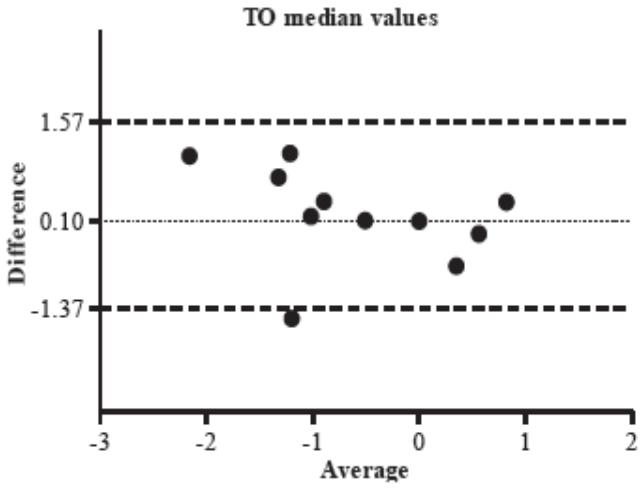

Fig. 1. Bland-Altman plot of TO median values.

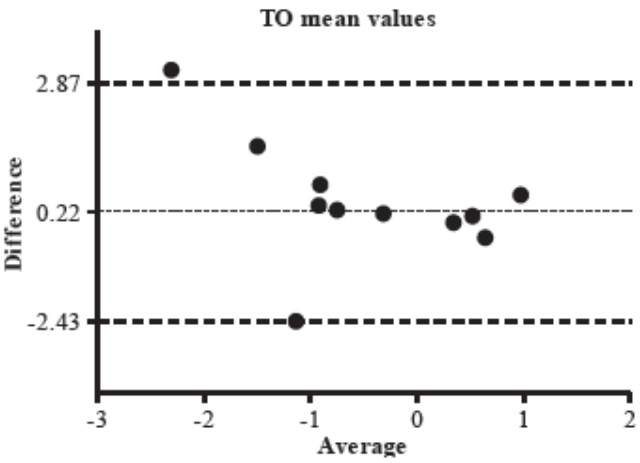

Fig. 2. Bland-Altman plot of TO mean values.

This might also be the explanation why the risk predictive power of heart-rate turbulence appears to be superior to that of heart-rate variability [26].

In our study the results suggest that median values of heart rate turbulence indexes might be preferred to commonly suggested mean values [36] for different reasons. In fact, since TO and TS showed highly skewed non-gaussian distribution, they are better described by median than mean values.

There were also observed significant correlations between the parameters of HRT, especially in the component TS (which reflects the gradual lengthening of the RR interval after the initial acceleration) and the parameters of the analysis of heart rate variability, which are the prevalent expression of vagal modulation, such as SDNN, pNN50 and RMSSD, with both indices of baroreflex sensitivity.

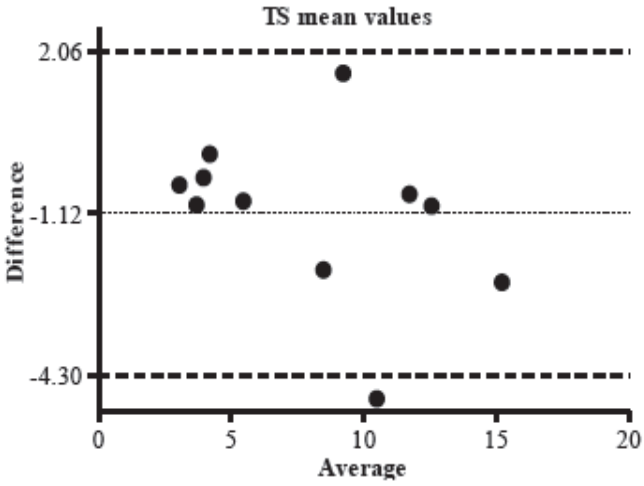

Fig. 3. Bland-Altman plot of TS median values

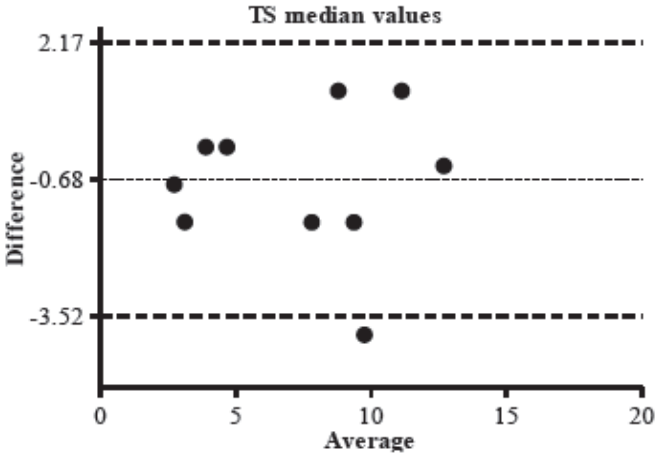

Fig. 4. Bland-Altman plot of TS mean values.

The autonomic changes induced by a ventricular premature beat initially depend on the reduction of the pressure pulse and the subsequent brief but significant baroreceptor deactivation.

The results are an initial sympathetic activation and vagal inhibition which is responsible for the shortening of the first sinus cycle, the extent of which is measured by the TO parameter.

Subsequently, as a result of the post-extrasistolic enhancement, it happens a sympathetic inhibition and an increase of vagal efferent that determines the progressive lengthening of the duration of RR intervals, until the achievement of the values observed before the ventricular exstasystole.

Therefore, the HRT is a non-invasive method which seems particularly suitable for the study of neural mechanisms of control in patients with cardiomyopathy characterized by a high incidence of ventricular premature beats and preserved pump function. It is well known that the ventricular extrasystoles constitute a disturbing element in the analysis of heart rate variability. Often, the Holter recording need editing that not only takes time, but can alter the meaning of the sequence of cardiac cycles.

As suggested by Schmidt et al. [26], although it is plausible to expect the cardiac autonomic status to influence heart-rate turbulence, it is also plausible to expect the physiological background of the turbulence to be different from that of heart-rate variability, which reflects, partly, the modulations of the cardiac autonomic status.

Long-term heart-rate variability probably mostly reflects autonomic responses to environmental and external stimuli that activate a broad variety of physiological reflexes. By contrast heart-rate turbulence is a phenomenon triggered by a minimum endogenous stimulus to which the reflex responses are possibly more organised and systematic.

Although TO showed narrower limits of agreements than TS, the latter can be considered much more reproducible than the former.

Practically algorithms for evaluation of heart rate turbulence indexes cannot exclude singles TO and TS outliers values and the median is much less sensitive than mean to outliers.

Moreover, by a methodological point of view the median TO and TS values showed higher reproducibility than means, which exhibit quite double bias and limit of agreement values.

By considering the data showed in Table 3 mean TO values were between -0.60 and -0.38%, while mean TS values were between 7.44 and 8.57 ms/RR.

Studies in normal healthy volunteers [45-48] reported mean TO values ranged from -2.7% to -2.3% and mean TS ranged from 11.0 to 19.2 ms/RR interval. Thus for TO, the difference between means, from Normal to heart failure patients, is of about 2%, which is just less than 1.5 times the standard deviation of bias reproducibility error of 1.35 shown in Table 4.

VIII. Conclusions

Heart rate turbulence is going to have wide and interesting clinical applications, showing very high prognostic value in several cardiac diseases. The importance of reproducibility data knowledge about this technique is at the base of a more accurate use of the information related to the heart rate turbulence indexes.

Differently for TS, the difference between means, from Normal to heart failure patients, is of about 7 ms/RR, which is at least more than 4 times the standard deviation of bias reproducibility error of 1.62 shown in Table 4. Consequently, the estimation of TS can be considered to show a sufficient degree of reproducibility, in contrast to what happens for TO.

Moreover, the data on Bland-Altman plots did not show any difference.

In fact, the variability between the two measures was consistent across the graph and the scatter around the bias line was unrelated to their average.

Preliminary results of this paper suggest the use of heart rate turbulence indexes median values instead of mean values and a reliability of the turbulence slope that appears greater than the turbulence onset index.

Data on the predictive value of heart rate turbulence in patients with heart failure remain limited. Data on the predictive value of heart rate turbulence in patients with heart failure remain limited.

The majority of data related abnormal heart rate turbulence with progression of disease [29-31, 33].

In the Marburg Study [30] turbulence onset was found a significant predictor of transplant free survivals in 242 patients with idiopathic cardiomyopaty. In a study of Koyama [30] including 50 patients with heart failure (both of ischemic and idiopathic etiology) abnormal turbulence slope defined as > 3

ms/RR was predictive for progression of heart failure including deaths and hospitalizations due to CHF worsening.

These observations were confirmed by data from UK Heart Study in patients with mild to moderate heart failure where abnormal turbulence slope was found to be an independent risk predictor of death due to decompensated heart failure [33].

Future extension of the work should be addressed to perform similar reproducibility study toward wider and different cardiac pathological populations and to confirm the higher value of the turbulence slope index.

IX. References

[1] Rich MW. Heart failure in the 21st century: a cardiogeriatric syndrome. *J Gerontol*, vol. 56, 2001 pp. M88-M96.

[2] Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation*, vol. 127, N° 1, 2013, pp. e6-e245.

[3] Matsushita K, Blecker S, Pazin-Filho A, Bertoni A, Chang PP, Coresh J, Selvin E. The association of hemoglobin A1c with incident heart failure among people without diabetes: the Atherosclerosis Risk in Communities study. *Diabetes*, vol. 59, 2010 pp. 2020–2026.

[4] Roger VL, Weston SA, Redfield MM, Hellermann-Homan JP, Killian J, Yawn BP, Jacobsen SJ. Trends in heart failure incidence and survival in a community-based population. *JAMA*, vol. 292, 2004 pp. 344–350.

[5] Levy D, Kenchaiah S, Larson MG, Benjamin EJ, Kupka MJ, Ho KK, Murabito JM, Vasan RS. Long-term trends in the incidence of and survival with heart failure. *N Engl J Med*, vol. 347, 2002, pp. 1397–1402.

[6] Barker WH, Mullooly JP, Getchell W. Changing incidence and survival for heart failure in a well-defined older population, 1970-1974 and 1990- 1994. *Circulation*, vol. 113, 2006, pp. 799–805.

[7] Chen J, Normand SL, Wang Y, Krumholz HM. National and regional trends in heart failure hospitalization and mortality rates for Medicare beneficiaries, 1998-2008. *JAMA*, vol. 306, 2011 pp. 1669–1678.

[8] Eckberg DL, Drabinsky M, Braunwald E. Defective cardiac parasympathetic control in patients with heart disease. *N Engl J Med*, vol. 285, 1971 pp. 877–883.

[9] Porter TR, Eckberg DL, Fritsch JM, Rea RF, Beightol LA, Schmedtje JF Jr, Mohanty PK. Autonomic pathophysiology in heart failure patients. Sympathetic–CholinergicInterrelations. *J Clin Invest*, vol. 85, 1990, pp. 1362–1371.

[10] Ferguson DW, Abboud FM, Mark AL. Selective impairment of baroreflex-mediated vasoconstrictor responses in patients with ventricular dysfunction. *Circulation*, vol. 679, 1984, pp. 451–460.

[11] Creager MA, Creager SJ. Arterial baroreflex regulation of blood pressure in patients with congestive heart failure. *J Am Coll Cardiol*, vol. 23, 1994 pp. 401–405.

[12] Mortara A, La Rovere MT, Pinna GD, Prpa A, Maestri R, Febo O, Pozzoli M, Opasich C, Gavazzi L, Arterial baroreflex modulation of heart rate in chronic heart failure: clinical and hemodynamic correlates and prognostic implications. *Circulation*, vol. 96, No. 10, 1997, pp. 3450-8.

[13] Frenneaux MP. Autonomic changes in patients with heart failure and in post-myocardial infarction patients. *Heart*, vol. 90, 2004, pp. 1248–1255.

[14] Cygankiewicz I, Zareba W, de Luna AB. Prognostic value of Holter monitoring in congestive heart failure. *Cardiol J*, vol 15, N° 4, 2008, pp. 313-23.

[15] Corbi GM, Carbone S, Ziccardi P, Giugliano G, Marfella R, Nappo F, Paolisso G, Esposito K, Giugliano D. FFAs and QT intervals in obese women with visceral adiposity: effects of sustained weight loss over 1 year. *J Clin Endocrinol Metab*, vol. 87, N° 5, 2002 pp. 2080-3.

[16] Cusenza M, Accardo A, D'Addio G, Corbi G, Relationship between fractal dimension and power-law exponent of heart rate variability in normal and heart failure subjects. *Computers in Cardiology*, vol. 37, 2010, pp. 935-938.

[17] D'Addio G, Cesarelli M, Romano M, Accardo A, Corbi G, Maestri R, La Rovere MT, Bifulco P, Ferrara N, Rengo F, Correlation between fractal behavior of HRV and neurohormonal and functional indexes in chronic heart failure. *IFMBE Proceedings*, vol. 25, 2010, pp. 53-56.

[18] Porta A, D'Addio G, Corbi G, Maestri R, Pinna GD, Circadian variations of short-term heart period irreversibility in healthy and chronic heart failure patients. *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, 2008, pp. 2116-2119.

[19] D'Addio G, Accardo A, Corbi G, Ferrara N, Rengo F, Functional Correlates of Fractal Behavior of HRV in COPD Patients. *IFMBE Proceedings*, vol. 25, 2010, pp. 261-264

[20] D'Addio G, Corbi G, Rengo G, Russo G, Pinna GD, Maestri R, Ferrara N, Rengo F, Poincaré plot analysis of heart rate variability in stroke patients. *IFMBE Proceedings*, vol. 11, 2005, pp. 632-635.

[21] D'Addio G, Corbi G, Accardo A, Russo G, Ferrara N, Mazzoleni Mc, Princi T, Fractal behaviour of heart rate variability reflects severity in stroke patients. *Studies in Health Technology and Informatics*, vol. 150, 2009, pp. 794-798.

[22] D'Addio G, Accardo A, Corbi G, Russo G, Pinna Gd, Ferrara N, Rengo F, Effects of Stroke Localization on Nonlinear Indexes of HRV. *Computers in Cardiology*, vol. 33, 2006, pp. 621-624.

[23] Accardo A, D'Addio G, Corbi G, Russo G, Ferrara N, Rengo F, Pinna GD, Fractal analysis of heart rate variability in stroke patient. *Biomedizinische Technik*, vol. 51, 2006, pp. A1-A10.

[24] D'Addio G, Pinna GD, Maestri R, Corbi G, Ferrara N, Rengo F, Quantitative Poincare Plots Analysis Contains Relevant Information Related to Heart Rate Variability Dynamics of Normal and Pathological Subjects. *Computers in Cardiology*, vol. 31, 2004, pp. 457-460,

[25] D'Addio G, Pinna GD, Cesarelli M, Maestri R, La Rovere MT, Romano M, Corbi G, Ferrara N, Rengo F. Neurohormonal and functional correlates of linear and poincare' plot indexes of heart rate variability in heart failure patients. *Eur J Heart Fail*, vol. 9, 2010, pp. S1.

[26] Schmidt G, Malik M, Barthel P, Schneider R, Ulm K, Rolnitzky L, Camm AJ, Bigger JT Jr, Schömig A, Heart-rate turbulence after ventricular premature beats as a predictor of mortality after acute myocardial infarction. *Lancet*, vol. 353, No 9162, 1999, pp. 1390-6.

[27] Berkowitsch A, Zareba W, Neumann T, Erdogan A, Nitt SM, Moss AJ, Pitschner HF. Risk stratification using heart rate turbulence and ventricular arrhythmia in MADIT II: usefulness and limitations of a 10-minute holter recording. *Ann Noninvasive Electrocardiol*, vol. 9, N° 3, pp.270-9.

[28] Cygankiewicz I, Zareba W, Vazquez R, Vallverdu M, Cino J, Cinca J, Almendral J, Gonzalez Juanatey JR, Macaya C, Valdes M, Caminal P, Bayes de Luna A. Relation of heart rate turbulence to severity of heart failure. *Am J Cardiol*, vol. 98, N° 12, 2006, pp. 1635-40.

[29] Cygankiewicz I, Zareba W, Vazquez R, Vallverdu M, Gonzalez-Juanatey JR, Valdes M, Almendral J, Cinca J, Caminal P, de Luna AB; Muerte Subita en Insuficiencia Cardiaca Investigators. Heart rate turbulence predicts all-cause mortality and sudden death in congestive heart failure patients. *Heart Rhythm*, vol. 5, N° 8, 2008, pp. 1095-1102.

[30] Grimm W, Schmidt G, Maisch B, Sharkova J, Müller HH, Christ M. Prognostic significance of heart rate turbulence following ventricular premature beats in patients with idiopathic dilated cardiomyopathy. *J Cardiovasc Electrophysiol*, vol. 14, 2003, pp. 819–824.

[31] Koyama J, Watanabe J, Yamada A, Koseki Y, Konno Y, Toda S, Shinozaki T, Miura M, Fukuchi M, Ninomiya M, Kagaya Y, Shirato K. Evaluation of heart-rate turbulence as a new prognostic marker in patients with chronic heart failure. *Circ J*, vol. 66, N° 10, 2002, pp. 902-7.

[32] Miwa Y, Ikeda T, Sakaki K, Miyakoshi M, Ishiguro H, Tsukada T, Abe A, Mera H, Yusu S, Yoshino H. Heart rate turbulence as a predictor of cardiac mortality and arrhythmic events in patients with dilated cardiomyopathy: a prospective study. *J Cardiovasc Electrophysiol*, vol. 20, 2009, pp. 788–795.

[33] Moore RK, Groves DG, Barlow PE, Fox KA, Shah A, Nolan J, Kearney MT. Heart rate turbulence and death due to cardiac decompensation in patients with chronic heart failure. *Eur J Heart Fail*, vol. 8, 2006, pp. 585–590.

[34] Voss A, Baier V, Schirdewan A, Leder U, Physiological hypotheses on heart rate turbulence, in *Dynamic Electrocardiography*, Malik M, Camm AJ editors, Oxford: Blackwell Publishing, 2002, pp. 203–10.

[35] D'Addio G, Cesarelli M, Corbi G, Romano, M, Furgi G, Ferrara N, Rengo F, Reproducibility of heart rate turbulence indexes in heart failure patients. *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, 2010, pp. 2573-2576.

[36] Bauer A, Malik M, Schmidt G, Barthel P, Bonnemeier H, Cygankiewicz I, Guzik P, Lombardi,F, Mu□ller A, Oto A, Schneider R, Watanabe M, Wichterle D, Zareba W, Heart rate turbulence: standards of measurement, physiological interpretation, and clinical use: International Society for Holter and Noninvasive Electrophysiology Consensus. *J Am Coll Cardiol*, vol. 52, No. 17, 2008, pp. 1353-65.

[37] D'Addio G, Cesarelli M, Maestri R, Furgi G, La Rovere MT, Ferrara N, Rengo F, Pre-Ectopic Vagal Tone Affects Heart Rate Turbulence Slope in Heart Failure. *Computing in Cardiology*, vol. 39, 2012, pp. 937-940.

[38] Kleiger RE, Bigger JT, Bosner MS, Chung MK, Cook JR, Rolnitzky LM, Steinman R, Fleiss JL, Stability over time of variables measuring heart rate variability in normal subjects. *Am J Cardiol*, vol. 68, No. 6, 1991, pp. 626-30.

[39] MATLAB® for Windows, The MathWorks, Inc. Natick, MA USA, http://www.mathworks.com

[40] Cygankiewicz I, Wranicz JK, Bolinska H, Zaslonka J, Zareba, Relationship between heart rate turbulence and heart rate, heart rate variability, and number of ventricular premature beats in coronary patients. *J Cardiovasc Electrophysiol*, vol. 15, 2004, pp.731–7.

[41] Bland JM, Altman DG, Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, vol. 1, No. 8476, 1986, pp. 307-10.

[42] Altman DG, Bland JM, Measurement in medicine: the analysis of method comparison studies. *The Statistician*, vol. 32, 1983, pp. 307-317.

[43] British Standards Institution Precision of Test Methods. I: Guide for the Determination and Reproducibility for a Standard Test Method London, U.K., BS 5497, Part 1; 1976, pp. 54-97.

[44] GraphPad Prism for Windows, GraphPad Software, San Diego California USA, www.graphpad.com

[45] Grimm W, Sharkova J, Christ M, Schneider R, Schmidt G, Maisch B, Heart rate turbulence following ventricular premature beats in healthy controls. *Ann Noninvasive Electrocardiol*, vol. 8, No. 2, 2003, pp. 127-31.

[46] Lindgren KS, Mäkikallio TH, Seppänen T, Raatikainen MJ, Castellanos A, Myerburg RJ, Huikuri HV, Heart rate turbulence after ventricular and atrial premature beats in subjects without structural heart disease. *J Cardiovasc Electrophysiol*, vol. 14, No. 5, 2003, pp. 447-52.

[47] Diaz JO, Castellanos A, Moleiro F, Interian A, Myerburg RJ, Relation between sinus rates preceding and following ectopic beats occurring in isolation and as episodes of bigeminy in young healthy subjects. *Am J Cardiol*, vol. 90, No. 3, 2002, pp. 332-5.

[48] Tuomainen P, Peuhkurinen K, Kettunen R, Rauramaa R, Regular physical exercise, heart rate variability and turbulence in a 6-year randomized controlled trial in middle-aged men: the DNASCO study. *Life Sci*, vol. 77, No. 21, 2005, pp. 2723-34.