Search for disease-specific cardiovascular reactivity patterns: developing the methodology

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ABSTRACT

Aberrations of CVR (cardiovascular reactivity), an expression of autonomic function, lack specificity for a particular disorder. Recently, a CVR pattern particular to chronic fatigue syndrome has been observed. In the present study, we aimed to develop methodologies for assessing disease-specific CVR patterns. As a prototype, a population of 50 consecutive patients with FMF (familial Mediterranean fever) was studied and compared with control populations. A 10 min supine/30 min head-up tilt test with recording of the heart rate and blood pressure or the pulse transit time was performed. Five studies were conducted applying different methods. In each study, statistical analysis identified independent predictors of CVR in FMF. Based on regression coefficients of these predictors, a linear DS (discriminant score) was computed for every subject. Each study established an equation to assess CVR, calculate DS for FMF and determine the sensitivity and specificity of the DS cut-off. In each of the five studies, abnormal CVR was observed in FMF patients. The best accuracy (88% sensitivity and 90.1% specificity for FMF) was obtained by a method based on beat-to-beat heart rate and pulse transit time recordings. Data was processed by fractal and recurrence quantitative analysis with recordings in FMF patients compared with a mixed control population. Identification of disease-specific CVR patterns was possible with the methodologies described in the present study. In FMF, disease-specific CVR may be explained by the interplay between neuroendocrine loops specific to FMF with cardiovascular homoeostatic mechanisms. Recognition of disease-specific CVR patterns may advance the understanding of homoeostatic mechanisms and have implications in clinical practice.

INTRODUCTION

The origin of the present study lies a few years back in the unexpected finding of a specific CVR (cardiovascular reactivity) pattern in patients with CFS (chronic fatigue syndrome) [1]. This observation was validated in five studies by using different methodologies [2–6].

Alterations in autonomic nervous system activity, i.e. dysautonomia, have been noted in a variety of rheumatic disorders, including rheumatoid arthritis [7],

Key words: cardiovascular reactivity, fractal analysis, familial Mediterranean fever, recurrence plot analysis, tilt test.

Abbreviations: BP, blood pressure; CFS, chronic fatigue syndrome; CVR, cardiovascular reactivity; DS, discriminant score; FD, fractal dimension; FMF, familial Mediterranean fever; DS-FMF, DS for FMF patients; FRAS, fractal and recurrence analysis-based score; HIS, haemodynamic instability score; HR, heart rate; HUTT, head-up tilt test; PTT, pulse transit time; RQA, recurrence quantification analysis.

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systemic lupus erythematosus [8], Sjogren’s syndrome [9], systemic sclerosis [10], ankylosing spondylitis [11], mixed connective tissue disease [10], fibromyalgia [12] and CFS [5]. The potential role of dysautonomia in the pathogenesis of rheumatic disorders has been suggested [13]. A recent study suggested that patients affected by FMF (familial Mediterranean fever) may have dysautonomia [14]. A portion (20 %) of FMF patients had an abnormal CVR recognized on postural challenge, including vasodepressor and cardioinhibitory reactions, orthostatic hypotension and the postural tachycardia syndrome. This dysautonomia was clinically hidden, but uncovered on postural challenge, and was not the result of amyloidosis in the patients under observation [14].

In the clinical setting, autonomic nervous system activity is assessed by surrogate methods, chiefly CVR. The fast response of BP (blood pressure) and HR (heart rate) to acute stimuli is under autonomic nervous control. Therefore BP and HR measurements during orthostatic challenge can be used as one measure of cardiovascular autonomic activity providing there is no evidence of heart failure, venous insufficiency or hypovolaemia [15,16]. Additional methods employed for the study of CVR are 24 h ambulatory monitoring to measure short- and long-term variability in BP and HR. In the laboratory, CVR is studied under physical, cold pressor or postural challenge, lower-body negative pressure, physical exercise or combined mental and physical challenges [17–19]. Fundamentally, these techniques measure BP and HR changes under diverse conditions and compare them with baseline values. To best achieve this purpose, attention has to be paid not only to average values of BP and HR, but also to fluctuations in the measurements around the means. The S.D. supplies data on the signal dispersion around the mean, but does not provide information on the patterns that characterize the variability of the signal over a period of time. This led to development of other methods for evaluating CVR, among which spectral analysis has been widely used [20].

In fact, autonomic cardiovascular modulation is characterized by a high degree of non-linearity between external stimuli and cardiovascular response [19]. Other methods have been proposed in an attempt to address the issue of non-linear dynamics of the cardiovascular responses. These include fractal analysis [21,22], RQA (recurrence quantification analysis) [23,24] and multivariate models that consider the relationship between two or more cardiovascular signals [19]. It has been suggested that joint quantification of BP and HR fluctuations and utilization of non-Euclidian mathematical analysis may provide more reliable information on CVR [19]. Recently, we developed a method to assess CVR while applying the above principles, including correlating the BP and HR fluctuations, by using common statistical as well as fractal analysis [5,6]. Introduction of these novel analytical methods in the processing of HR and BP recordings has permitted the description of specific disease-related CVR patterns for the first time [6].

Previous observations utilizing the HUTT (head-up tilt test) had detected clinically silent dysautonomia in FMF patients by utilizing only classical endpoints, such as vasovagal reactions and postural tachycardia [14]. We hypothesized that the application of new analytical methods in processing HR and BP recordings could render more data on dysautonomia in FMF. By working to improve the methodology, as applicable to FMF, we aimed to find what parameters are necessary to identify a disease-specific CVR and to answer the following questions: is an abnormal CVR detectable in FMF patients and, if present, is the abnormal CVR in FMF specific for this condition?

METHODS

All participants gave informed consent and the study was approved by our institution’s Human Research Committee. All patients were fully ambulatory at the time of the study. The patients were not taking medication for at least 2 weeks before the study. Technicians carrying out the HUTT were informed of the patients’ diagnoses, but did not know of the intention to compare between the groups. Data of earlier studies, which utilized different analyses and were based upon somewhat different dependent measures, were revised, expanded and processed according to the latest methodology [2,5].

Study population

The FMF patient group included 50 consecutive adult patients with FMF referred from a rheumatology clinic. The entry criteria for the patients were as follows: (i) age between 20–60 years; (ii) fulfilment of the criteria for the diagnosis of FMF [25]; (iii) last attack of FMF ending at least 7 days prior to the HUTT; (iv) absence of active co-morbidity, except amyloidosis; and (v) absence of any medication that might affect autonomic nervous system function, including any type of sleeping pills, tranquillizers or antidepressants, for at least 1 month prior to the study. The patients’ average age was 30.1 (S.D., 9.2) years, and 68 % were male. None had a history of syncope. The subjects had normal findings on physical examination, chest X-rays and ECG. Various comparison populations were designated for each of five intended substudies. The pool of subjects for comparison included the following groups. (a) Healthy subjects (n = 59) recruited from the hospital staff to participate in the study. Subjects were eligible if they were asymptomatic, did not take medication in the 15 days prior to the present study and had normal findings on physical examination, routine laboratory tests, chest X-rays and ECG. Their average age was 30.1 (S.D., 9.2) years, and 68 % were male. (b) CFS patients (n = 40) were referred from a CFS clinic
for evaluation by HUTT. All patients met the Centers of Disease Control and Prevention definition criteria of CFS [26]. The subjects’ mean age was 30.8 (16.1) years, and 65% were women. The median duration of illness was 17.1 months (range, 7 months–4 years). Amyloidosis was diagnosed in five patients. (c) Patients with non-CFS chronic fatigue (n=73), similar to CFS patients, complained of fatigue of new onset, not a result of ongoing exertion, not substantially alleviated by rest, associated with substantial reduction in previous levels of activity, and lasting 3 months or more, but they did not otherwise meet the definition criteria of CFS. The subjects’ mean age was 31.9 (9.7) years, and 66.6% were women. Median duration of illness was 7 months (range, 3–13 months). (d) Patients with fibromyalgia (n=41) were referred from a rheumatology clinic. The diagnosis of fibromyalgia was based on criteria of the American College of Rheumatology for fibromyalgia [27]. Sixteen subjects who also reported fatigue, but did not primarily meet the diagnosis of CFS, were included. The patients’ median age was 30 (range, 22–67) years, and 68% were women. (e) Patients with neurally mediated syncope (n=58) were referred for HUTT in order to evaluate syncope of unknown cause. All subjects had two or more syncopal or presyncopal spells during the previous 3 months. The subjects’ mean age was 29.3 (18.2) years, and 66.6% were women. The diagnosis of neurally mediated syncope [16] was eventually established. (f) Patients with psoriatic arthritis (n=20) fulfilled the diagnostic criteria of psoriatic arthritis [28]. The patients’ average age was 31 years (5.8), and 63.2% were women. (g) Patients with rheumatoid arthritis (n=10) fulfilled the revised criteria for the classification of rheumatoid arthritis [29]. The patients’ average age was 42.2 years (6.3), and 90% were women.

HUTT protocol

The protocol of the HUTT was based on the 10 min supine/30 min HUTT as described previously [1]. Manual BP readings were taken by a physician certified in the BP measurement technique according to American Heart Association recommendations [30]. We favoured the mercury column sphygmomanometer (Baumanometer, standby model 0661-0250), since this is the standard non-invasive method for BP measurement [31]. HR measurements were recorded on an electrocardiographic monitor. The patient lay in a supine position on the tilt table, secured to the table at the chest, hips and knees with adhesive girdles. The cuff of the BP recording device was attached to the left arm, which was supported at heart level at all times during the study. Measurements in the supine position were recorded at 5 min intervals. The last supine BP was the median of three consecutive measurements taken at 10 min. The table was then gently tilted head-up to an angle of 70°. The duration of the tilt was 30 min. During the initial 5 min of tilt, measurements were obtained at 1 min intervals, followed by readings every 5 min. When dizziness or faintness occurred, repeated measurements were taken at 30 s intervals. In the event of a loss of consciousness, the test was discontinued. Postural tachycardia was diagnosed when a HR increment of 30 beats/min or greater appeared during the first 5 min of tilt in the absence of orthostatic hypotension. Orthostatic hypotension was diagnosed when a fall of systolic BP of at least 20 mmHg, or a fall of diastolic BP of at least 10 mmHg, occurred during the first 3 min of tilt associated with an increase of HR of at least 10 beats/min compared with the last supine measurement. Vasodepressor reaction was diagnosed when a BP fall of a similar degree occurred after the initial 3 min of the tilt associated with an increase of HR of at least 10 beats/min. Cardioinhibitory syncope was diagnosed when a fall in BP of a similar degree occurred after the initial 3 min of tilt and was not associated with an increase in HR [16].

Calculation of DS-FMF [DS (discriminant score) for FMF patients] based on the analysis of HR and BP

The relative changes of BP and HR were calculated (Figure 1). The systolic and diastolic BP differences were defined as the differences between individual BP values measured during HUTT and the last supine BP value (BPn3) divided by the last supine BP value as follows:

\[
\text{BP difference} = \frac{\text{BP(n1...n13) − BPn3}}{\text{BPn3}}
\]

where BPn is the BP measurement number. The means and S.D. of the current values of the systolic and diastolic BP differences were calculated. HR differences were calculated similarly. The absolute systolic and diastolic BP differences and HR differences were computed by transforming positive and negative BP differences into positive values. The current and absolute BP differences and HR differences were also represented graphically in time curves and the fractal dimensions of the time curves were calculated [1]. The mean and S.D. systolic and diastolic BP difference were calculated for each subject and termed ‘current’ BP difference [SYST-DIFF-c-AVG and SYST-Diff-c-SD (average and S.D. respectively, of the systolic BP changes – current values); DIAST-DIFF-c-AVG and DIAST-Diff-c-SD (average and S.D. respectively, of the diastolic BP differences – current values)]. The BP differences were also represented graphically in time curves. These Figures were constructed in a fixed template on Microsoft Excel graphics. The images were loaded in the computerized image analyser Benoit Version 1.3 (Trusoft International, St. Petersburg, FL, U.S.A.). The FD (fractal dimension) was automatically assessed by using the box-counting method.

Absolute systolic and diastolic BP differences were calculated by transforming positive and negative BP
Figure 1 An example of the calculation of DM-FMF based on the analysis of HR and BP

(A) Systolic, diastolic BP, and HR values of a patient with FMF taken throughout the HUTT. Supine measurements at 1, 5 and 10 min of recumbence correspond to n1 to n3, whereas head-up measurements at min 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 of tilt correspond to n4 to n13 respectively. From the measured values, the relative changes of BP and HR were calculated, according to the equation: BP difference \(= \frac{\text{BP}(n1...n13) - \text{BP}n3}{\text{BP}n3}\). Absolute values were then obtained by converting all results to positive numbers. Systolic and diastolic BP differences are shown as absolute (a) values. The genuine BP and HR values were used to calculate the DIAS-AVG and HR-AVG. The systolic BP changes were used to calculate the SYST-DIFF-a-SD. The fourth independent predictor of DS is the DIAS-DIFF-a-FD, and it was processed from the time-curve of the diastolic BP differences (B) by a fractal analysis program. (C) The four independent predictors were applied to the equation to compute the DS-FMF3.

For multivariate analysis, forward stepwise logistic regression was applied to evaluate independent predictors of the CVR in FMF compared with CVR variables of control patients. Based on the regression coefficients (slopes and intercept) of these predictors, a linear DS was computed for each subject. The best cut-off was established between the DS-FMF compared with DS of controls.

Calculation of DS-FMF based on fractal analysis and RQA of HR and PTT (pulse transit time)

We have recently developed [5] a method for the study of CVR based on fractal analysis and RQA of HR and PTT during the course of a HUTT, whereby the FRAS (fractal and recurrence analysis-based score) is calculated. The method is described in detail elsewhere [5]. The cuff of the BP recording device was attached to the left arm, which was supported at heart level at all times during the study. The right forearm and hand were supported by a cast and suspended with a sling around the patient’s neck. The fingers pointed to the mid-axillary line at the level of the fourth intercostal space. The photoelectric sensor of the photoplethysmograph was placed on the distal phalanx of the second or third finger. The ECG and photoplethysmograph were recorded on a Datex-Engstrom Cardiocap™ II instrument (Datex Instrumentation, Helsinki, Finland), connected to the Biopac MP 100 data acquisition system (Santa Barbara, CA, U.S.A.). PTT was computed automatically on the AcqKnowledge software (Biopac), and the tracings were displayed continuously. The computer program identified PTT as the time interval between the peak of the electrocardiographic R wave and the peak of the
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pressure wave at the finger, as measured by the pulse plethysmograph. A sample rate of 500 data points/s provided 1/500 Hz resolution for the HR and PTT measurements. Measurements were acquired in the supine position over a 10 min period. The table was then gently tilted head-up to an angle of 70° and the acquisition continued for a total of 600 cardiac cycles (usually 5–10 min). The RR intervals on ECG recordings and the corresponding PTT values were automatically computed with the AcqKnowledge software. Four sets of values were obtained each comprising approx. 600 measurements, the HR supine, PTT supine, HR tilt and PTT tilt. The measurements were reviewed and edited to screen out artefacts.

The HR and PTT measurements in the supine and tilt phases were processed separately by three mathematical methods: general statistics, fractal analysis and recurrence plot analysis, as described in detail elsewhere [5]. For the general statistics, the average, S.D., minimum, maximum, variance, skewness and kurtosis were calculated. For fractal analysis, time series of 500–600 consecutive edited measurements, either HR or PTT, were loaded into the Benoit Version 1.3 analyser (Trusoft International). Time curves were constructed. FD of the time curve was calculated with the aid of four different methods: R/S, roughness length, variogram and wavelets analysis. RQA was performed with the Visual Recurrence Analysis computer program version 4.2 developed by Eugene Kononov [24]. The RQA variables computed were the recurrence, determinism, ratio, entropy, maxline, trend and spatio-temporal entropy. Thus four data sets were obtained in each patient, including HR and PTT in supine and tilt positions, together comprising the variables of CVR (23 x 4 = 92 variables).

Multivariate analysis was conducted, evaluating those variables that might function as independent predictors for the assessment of the cardiovascular activity at rest and CVR on postural challenge in the fibromyalgia group as distinct from the comparison group. Based on the regression coefficients (slopes and intercepts) of these predictors, an equation was determined to calculate the linear DS.

Substudies

In each substudy, an equation was proposed to calculate DS and to determine the sensitivity and specificity of the DS cut-off for FMF.

Substudy I

An equation that was originally developed for assessment of CVR in CFS was applied in substudy I for the assessment of the CVR in FMF patients.

Substudy II

An equation was specifically developed to assess CVR in FMF patients against healthy subjects.

Substudy III

An equation was specifically developed to assess CVR in FMF patients against a mixed group of control subjects.

Substudy IV

An equation was specifically developed to assess CVR in FMF against a larger mixed group of control subjects.

Substudy V

Fractal analysis and RQA of HR and PTT was utilized for processing the recordings. An equation was specifically developed to assess CVR in FMF patients against a large mixed group of control subjects.

Statistical analysis

Values are means (S.D.) unless otherwise stated. A two-tailed P value ≤0.05 was accepted as statistically significant. The predictive characteristics of DS (sensitivity and specificity) were calculated from the logistic model, using regression coefficients of the relevant independent variables. Receiver operator characteristic curve analysis was built using Wilcoxon’s method for detecting the best cut-off point of DS.

RESULTS

In FMF patients, postural tachycardia occurred in eight patients and vasovagal reactions (cardioinhibitory, vaso-depressor or mixed) occurred in five patients, necessitating the premature termination of HUTT in three instances. In healthy controls, there was no instance of postural tachycardia or vasovagal reaction, whereas, in the remaining 242 control patients, 83 had one or other of these reactions. In the group of FMF patients, the average systolic BP in the supine position was 117.4 (17.2) mmHg and 109.1 (16.6) mmHg on tilt; the average diastolic BP in supine position was 78.8 (11.7) mmHg and 78.6 (13.8) mmHg on tilt. The average HR in supine position was 69.8 (104) beats/min and 84.3 (13.6) beats/min on tilt.

In the subgroup of healthy controls, the average systolic BP in the supine position was 116.8 (9.1) mmHg and 109.5 (8.2) mmHg on tilt; the average diastolic BP in supine position was 69.3 (7.4) mmHg and 73.0 (7.1) mmHg on tilt. The average HR in supine position was 67.9 (9.2) beats/min and 77.1 (0.8) beats/min on tilt.

Substudy I

A method originally developed for assessment of CVR in CFS [1] [called HIS (haemodynamic instability score)] was applied in substudy I for the assessment of CVR in FMF patients:

\[
\text{HIS} = 64.3303 + (\text{SYS-DIFF-a-FD} \times -68.0135)
+ (\text{SYS-DIFF-c-SD} \times 111.3726)
+ (\text{HR-DIFF-c-SD} \times 60.4164)
\]
The recordings were determined in patients with FMF \((n = 50)\), CFS \((n = 40)\), non-CFS chronic fatigue \((n = 73)\), fibromyalgia \((n = 41)\), neurally mediated syncope \((n = 58)\) and essential hypertension \((n = 28)\) and healthy individuals \((n = 59)\). The average HIS values were \(-5.22 (4.44)\) in FMF patients, \(+2.54 (2.55)\) in CFS patients, \(-3.61 (3.95)\) in non-CFS fatigue, \(-3.1 (2.62)\) in fibromyalgia patients, \(-3.55 (3.38)\) in patients with syncope, \(-5.53 (2.24)\) in patients with essential hypertension and \(-3.13 (2.98)\) in healthy individuals. In FMF patients, HIS > -0.94 was observed in three out of 50 patients (6%). Although HIS values in CFS patients differed significantly from HIS values in other groups \((P < 0.0001)\), the HIS values in FMF patients did not differ significantly from HIS in healthy subjects.

**Substudy II**

In this analysis, DS was set to recognize CVR in FMF patients compared with CVR in healthy individuals (this is DS-FMF1). Recordings of 50 FMF patients and 23 age- and sex-matched healthy controls were evaluated. The best independent predictors of the CVR in FMF compared with healthy subjects were identified, and an equation was derived to calculate DS-FMF1:

\[
DS\text{-FMF1} = -5.58 + (0.15 \times HR\text{-AVG})
- (3.59 \times HR\text{-DIFF-a-SD})
\]

DS-FMF1 values were \(+5.83 (1.78)\) in FMF patients and \(-7.60 (5.41)\) in healthy individuals \((P = < 0.0001)\). The DS-FMF1 cut-off > 3.25 differentiated FMF from healthy subjects with 98.2 % sensitivity and 100 % specificity. Subsequently, the specificity of the DS-FMF1 cut-off > 3.25 was tested in additional groups: psoriatic arthritis \((n = 10)\), fibromyalgia \((n = 10)\), CFS \((n = 13)\) and non-CFS fatigue \((n = 12)\). The average DS-FMF1 values in these groups ranged from \(+5.2\) to \(+6.6\), which were comparable with values in FMF patients (Figure 2). The overall specificity of the DS-FMF1 cut-off was 35.7 %.

**Substudy III**

DM-FMF2 was set to recognize CVR in FMF patients compared with CVR in a mixed group of control subjects. The database of a previous study was re-evaluated \([14]\), and four groups of age- and sex-matched subjects (patients with FMF, psoriatic arthritis and fibromyalgia and healthy subjects), each comprising 20 subjects, were studied. Based on independent predictors, an equation was derived to calculate DS-FMF2:

\[
DS\text{-FMF2} = -2.26 + (25.68 \times SYS\text{-DIFF-a-SD})
+ (0.19 \times HR\text{-DIFF-c-SD})
\]

The DS-FMF2 cut-off > 0.34 had a 45 % sensitivity and 91.25 % specificity for FMF.

![Figure 2](image)

**Figure 2** DS-FMF1 values in FMF patients and control groups

The boxes contain the 50 % of values falling between the 25th and 75th percentiles, the horizontal line within the box represents the median value, and the whiskers are the lines that extend from the box to the highest and lowest values, excluding the outliers. Set to differentiate between the CVR in FMF and the reactivity in healthy subjects, the DS-FMF1 cut-off > 3.25 distinguished between FMF and healthy subjects with 98.2 % sensitivity and 100 % specificity. However, the specificity was unsatisfactory against other comparison populations. ARTH, arthritis; FM, fibromyalgia; F, non-CFS fatigue; H, healthy.

**Substudy IV**

The unsatisfactory sensitivity of DS-FMF2 motivated the expansion of the comparison group in sub-study IV. DS-FMF3 was set to recognize CVR in FMF patients compared with CVR of a larger mixed group of control patients. Six groups of age- and sex-matched subjects (patients with FMF, rheumatoid arthritis, psoriatic arthritis, fibromyalgia, CFS and non-CFS fatigue and healthy subjects), each comprising 20 subjects, were studied. BP and HR data of FMF patients were compared with the data from the mixed pool of patients using the equation:

\[
DS\text{-FMF3} = -3.82 + (DIAS\text{-DIFF-a-FD} \times 0.0078)
+ (DIAS\text{-AVG} \times 0.08) - (HR\text{-AVG} \times 0.04)
+ (SYST\text{-DIFF-a-SD} \times 27.17)
\]

The DS-FMF3 cut-off > 0.47 had 33.3 % sensitivity and 90.0 % specificity for FMF (Figure 3).

**Substudy V**

This substudy was based on fractal analysis and RQA of beat-to-beat recordings of HR and PTT, and DS-FMF4 was set to assess CVR in FMF patients against a large mixed group of control subjects. Six groups of subjects (patients with FMF \((n = 17)\), CFS \((n = 20)\), non-CFS fatigue \((n = 15)\), neurally mediated syncope \((n = 15)\) and psoriatic arthritis \((n = 19)\) and healthy individuals \((n = 20)\)), matched for age and gender, were examined. Multivariate analysis identified independent predictors of CVR in FMF against controls to be supine/PTT kurtosis.
by general statistics ($P < 0.0001$), tilt/HR minimum by general statistics ($P = 0.0002$), tilt/HR variance by general statistics ($P = 0.0001$), tilt/HR R/S by fractal analysis ($P = 0.0024$), tilt/HR average by general statistics, and tilt/PTT trend by RQA ($P < 0.0001$). Based on these predictors, DS-FMF4 was calculated according to the equation:

$$DS\text{-}FMF4 = -33.56 + (S_{\text{PTT, GKV}} \times 0.032) + (T_{\text{HR, GMI}} \times 0.13) + (T_{\text{HR, GVA}} \times 0.024) + (T_{\text{HR, R/S}} \times 11.21) + (T_{\text{PTT, GAV}} \times 14.77) + (T_{\text{PTT, TREN}} \times 0.018)$$

where $S_{\text{PTT, GKV}}$ is supine/PTT kurtosis, $T_{\text{HR, GMI}}$ is tilt/HR minimum, $T_{\text{HR, GVA}}$ is tilt/HR variance, $T_{\text{HR, R/S}}$ is tilt/HR R/S, $T_{\text{PTT, GAV}}$ is tilt/HR average, and $T_{\text{PTT, TREN}}$ is tilt/PTT trend.

The DS-FMF4 cut-off $> -0.27$ differentiated FMF from all other patients with 88% sensitivity and 90.1% specificity (Figure 4).

**DISCUSSION**

According to common understanding, patients with altered cardiovascular regulation may all be part of a single continuum of autonomic disturbances and the CVR is unrelated to a particular aetiology or nosological entity [32]. In contradistinction, recent studies suggest that disease-related CVRs may exist. A disease-related CVR phenotype has been described in CFS [1–6] and another phenotype in patients with neurally mediated syncope [6]. The present study showed that an abnormal CVR exists in the large majority of FMF patients and is disease-specific. This specific CVR pattern in FMF is exposed by a method based on fractal analysis and RQA of HR and PTT. In the FMF patient group, events of postural tachycardia and vasovagal reactions occurred in 26% of patients, in line with previous observations [14], whereas 88% of patients tested positive according to the proposed DS-FMF4 cut-off. All those who met classical criteria for dysautonomia were identified by the DS-FMF4 cut-off.

The previously described methods differ from common tests of CVR by applying novel techniques based on HIS and FRAS, designation of a wide range of control populations and attempting to identify a disease-related CVR phenotype. Processing of HR and BP (PTT) measurements was based on regular statistics as well as fractal analysis and RQA. Each mathematical method supplied a different dimension to the analysis of the time series of measurements. Fractal measurements differ from those used in regular Euclidean geometry [33]. FD represents a ‘self-similarity’ in dynamic behaviour over multiple scales of time. FD can be seen as the minimum number of underlying variables that are required to explain the signal as it appears. The lower the dimension, the simpler the signal. RQA is a relatively new analytical tool for the study of nonlinear dynamic systems. RQA is a technique originally developed by Eckman et al. [23] as a purely graphic tool to analyse dynamic processes. With RQA, one can graphically detect hidden patterns and structural changes in data and perceive similarities across the time series under study. In our present study, fractal and RQA measures contributed as independent predictors of CVR in FMF patients compared with control patients.

The search for methodologies for assessing diseasespecific CVR patterns is illustrated in the sequence of the five consecutive substudies. In substudy I, a method originally developed for assessment of the CVR in CFS [1], called HIS, and found to be specific in this disorder, was applied for the assessment of the CVR in FMF patients. HIS is a discriminant score specifically devised
to identify the disease-specific CVR pattern in CFS. Confirming expectations, HIS values in FMF patients differed significantly from HIS values in CFS patients and did not point to an abnormality in FMF.

In substudy II, DS was set to recognize CVR in FMF patients against CVR in healthy subjects. Indeed, 98.2% of FMF patients had an aberrant CVR when compared with healthy subjects, which is consistent with dysautonomia, given that confounding factors, such as heart failure, venous insufficiency or hypovolaemia, were excluded [16]. However, the overall specificity was poor when subsequently tested in additional groups, such as psoriatic arthritis, fibromyalgia, CFS and non-CFS fatigue.

Substudies III, IV and V were directed to answer the question of whether the abnormal CVR described in FMF is specific to this condition. As shown in substudy II, designation of the control group affects the specificity of DS. Therefore we hypothesized that utilization of a large heterogenic control population is needed to address the question of specificity. In substudies IV and V, large mixed pools of control patients were chosen, including a variety of clinical disorders, as well as healthy individuals. Specificity for FMF required that the technique could separate between clinically similar conditions. The best results were obtained in substudy V, which utilized a methodology including beat-to-beat assessment of PTT and HR and FRAS analysis. The proposed method recognized an FMF-related CVR with 88% sensitivity and 90.1% specificity (Table 1 and Figure 4).

In our attempt to refine the method for assessment of CVR, the following principles emerged. First, a method developed to assess CVR in one particular disorder is probably inappropriate and should not be applied to assess CVR in a different disorder. Secondly, beat-to-beat analysis of HR and PTT for assessment of CVR in FMF provided better results than HR and BP measurements at 5 min intervals. Only substudy V, which was based on the latter method, made it possible to distinguish a specific disease-related CVR in FMF patients. Thirdly, although intuitively obvious, it was demonstrated that selecting a heterogeneous large comparison group is essential for exposing a disease-related CVR. Fourthly, designation of the study group, its ethnic background, age limits, gender distributions, clinical variations and severity are essential requirements. An equation computed for assessment of DS-FMF matches a particular study population, but may be inadequate for FMF patients with a different ethnic background or presenting different clinical features.

The best results were obtained in substudy V. The proposed method recognized an FMF-related CVR with 88% sensitivity and 90.1% specificity. A subsequent study (J. E. Naschitz, M. Rozenbaum, E. Sabo and I. Rosner, unpublished work) validated the method described in the present investigation in substudy V. A second different group of 35 consecutive FMF patients, 21 healthy subjects and 21 patients with neurally mediated syncope was assessed. In this validation study, the sensitivity of the proposed equation for FMF was 61.9% and the specificity was 80%. Thus existence of a specific disease-related CVR in FMF patients was confirmed, although at a lower sensitivity than in the present study.

The mechanisms involved in disease-related phenotypes of CVR are not understood at the present time, but insight could come from studies into the pathophysiology of dysautonomia in general, and in rheumatic disorders in particular [34]. The conventional understanding that CVR is merely the result of arterial baroreflexes is simplistic and no longer tenable. Thus serotonin, aden- osine and opioids are additional triggers of the Bezold–Jarisch reflex, peripheral sympathetic afferents are directly activated by circulating mediators and higher nervous centres modulate the cardiovascular reflexes [34–37]. Any of these mechanisms can be involved to different degrees and modulate the particular phenotype of CVR linked to certain disease states. We hypothesize that disease-specific CVR patterns are determined by unique permutations in the cardiovascular, neuroendocrine and paracrine milieu in certain disorders. The nature of humoral mediators and cytokines in FMF which correlate with the disease-specific CVR pattern remains to be established. It is important to emphasize that not all groups of individuals have specific CVR patterns. In a previous study [6], it was not possible to define a consistent or specific CVR pattern in healthy individuals. We speculate that CVR in healthy individuals is heterogenic, not being modulated by a specific pathological mechanism into uniformity. A similar study conducted in patients with fibromyalgia ([16], and J. E. Naschitz, M. Rozenbaum, E. Sabo and I. Rosner, unpublished work) did not reveal a specific CVR pattern in the study patients. These data are more consistent with the concept that fibromyalgia is a common symptomatic framework rather than a separate disease entity and the patients’ CVR pattern is, accordingly, heterogenic.

The direct clinical meaning of the equations described in the present study is unclear. The existence of disease-specific CVR patterns may have implications in clinical practice. Recognition of the CFS reactivity phenotype has been found to be useful in supporting the clinical diagnosis of CFS and its numerical expression provided

<table>
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<th>Table I</th>
<th>Sensitivity and specificity of different methods in the recognition of CVR associated with FMF</th>
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<tr>
<td>Substudy</td>
<td>Equation</td>
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<tr>
<td>II</td>
<td>DS-FMF1</td>
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<tr>
<td>III</td>
<td>DS-FMF2</td>
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an objective criterion for monitoring the course of dysautonomia in CFS under treatment [38]. The fact that the CVR pattern in fibromyalgia appears to differ from the reactivity in CFS lends support to the notion that different pathophysiological pathways may be operative in these clinically similar entities. The clinical implications of finding a specific CVR pattern in FMF have not been explored. Potentially, identifying the FMF phenotype by HUTT in an atypical patient could strengthen the diagnosis of FMF in the appropriate clinical context, much as the finding of certain DNA mutations, whose immediate clinical significance is unclear, strongly favours the diagnosis of FMF without providing definitive proof.

The familiar HUTT can expose vasovagal reaction, postural tachycardia and orthostatic hypotension, syndromes which are not specific for a particular disease entity. In contradistinction, techniques like FRAS and HIS can describe disease-specific CVR patterns, which, although overlapping with classical features of dysautonomia, extend beyond them.

We are just beginning to learn about disease-related CVR phenotypes, developing methods to identify such phenotypes, seeking insight into the distinctive pathophysiological mechanisms and determining the importance of disease-related CVR phenotypes for clinical practice. Study of CVR in FMF is an example of such developments.

REFERENCES


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