Multiscale, multiorgan and multivariate complexity analyses of cardiovascular regulation

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Cardiovascular system complexity is confirmed by both its generally variegated structure of physiological modelling and the richness of information detectable from processing of the signals involved in it, with strong linear and nonlinear interactions with other biological systems. In particular, this behaviour may be accordingly described by means of what we call MMM paradigm (i.e. multiscale, multiorgan and multivariate). Such an approach to the cardiovascular system emphasizes where the genesis of its complexity is potentially allocated and how it is possible to detect information from it. No doubt that processing signals from multi-leads of the same system (multivariate), from the interaction of different physiological systems (multiorgan) and integrating all this information across multiple scales (from genes, to proteins, molecules, cells, up to the whole organ) could really provide us with a more complete look at the overall phenomenon of cardiovascular system complexity, with respect to the one which is obtainable from its single constituent parts. In this paper, some examples of approaches are discussed for investigating the cardiovascular system in different time and spatial scales, in studying a different organ involvement (such as sleep, depression and multiple organ dysfunction) and in using a multivariate approach via various linear and nonlinear methods for cardiovascular risk stratification and pathology assessment.

Keywords: cardiovascular system; multivariate approach; multiple organ disease; multiscale approach; risk stratification; cardiovascular genomics and proteomics

1. Introduction

The regulation of the cardiovascular system is based on a complex adjustment of various parts of the organism with respect to internal requirements, as well as to environmental influences. This comprehensive concept was earlier termed ‘homeostasis’ (Claude Bernard, 1813–1878), which means that diverse

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physiological mechanisms follow the common purpose to maintain the interior of
the organism stable. In the last two decades, concepts of nonlinear dynamics and
statistics were found to be essential for complex physiological control, leading to
the updated term ‘homeodynamics’. Nowadays, it is accepted that the organism
is a dynamically stable network of communicating elements from molecular,
cellular up to organ-level scales.

The objective of the present work is to review appropriate approaches
addressing those mechanisms. Multiple scales, multiple organs and multiple
variables are three essential aspects, which will be introduced and demonstrated
by representative application examples.

‘Multiscale’ approaches address the relationship between different scales such as
genotype and phenotype, which integrate the information obtained from genetic
mutation and the associated changes of protein, molecular, ion channel, cellular,
tissue and organ behaviour. New techniques of proteomics ($2a$) and the
associations between genotype and cardiovascular rhythms ($2b$) are of particular
interest. Another aspect of multiscale integration is the association between
different time scales, such as shown for the prognostic value of autonomic
information flow (AIF) between cardiac and cardiovascular control ($2c$).

The latter example leads to the aspect of ‘multiorgan’ integration. A primary
pathological disturbance and the resulting dysfunctions usually affect several
other organs within the entire organism and do not appear as isolated disorder.
Relevant examples are the associations between depression and cardiovascular
regulation ($3a$), between cerebral and cardiovascular functions during sleep
apnoea ($3b$), and between inflammation, infection and dysfunction of several
organs, which are reflected in particular heart rate rhythms ($3c$).

A resulting challenge is the assessment of multiple variables by ‘multivariate’
statistical analysis. Examples of survival studies in chronic heart failure (CHF)
patients are discriminant analysis and ROC curve methodology ($4a$) and Cox
regression models ($4b$). Another topic of multivariate analysis is nonlinear
associations (NLAs) between different signals, such as demonstrated for cross-
conditional entropy of electrical activity between different areas of the atrium in
connection with atrial fibrillation ($4c$).

2. Multiscale approaches to cardiovascular regulation

Traditionally, biological signal analysis is carried out at the level of the organ or
system to be investigated (i.e. electrocardiogram (ECG) signal, arterial blood
pressure, respiration and so on). It is very important to understand how to take
advantage of correlating this information with that obtained about the same system,
but at a different scale level, i.e. at the cellular level or even at the subcellular level
(e.g. analysing possible genetic correlates or typical patterns of proteins or even
DNA/RNA sequences). Biomedical engineering as a dedicated discipline may
strongly contribute to this multiscale information processing (Clancy & Rudy 1999).

Along this approach line, figure 1 (from Hunter et al. 2002) illustrates how an
important cardiac pathology, the long-QT syndrome, can be efficiently studied at
a different scale level: a mutation in a portion of gene SCN5A, which presents a
phenotype compatible with long-QT3 type, is known to produce an altered
function of Na$^+$ channels. Through a proper model that describes the functioning
of ventricular cells, it is possible to evidence that this alteration may induce a prolongation of QT duration, as detected on ECG tracing. This event is further correlated with an increased risk of ventricular tachyarrhythmias. Hence, the path is completed: from the genetic expression up to the disease manifestation (Clancy & Rudy 1999; Hunter et al. 2002; Priori et al. 2003). Many different signal processings and modellings are involved in this paradigmatic example. Integration along the various scales of observation may undoubtedly contribute to a better understanding of the complex pathophysiological correlates. Even if this sequence of passage from gene up to organ seems to be conceptually clear and promising, from a clinical standpoint, the relationships between genotype (possible genetic defect) and phenotype (manifestation of the related pathology) are not yet unambiguously determined. It is important to understand why in certain cases one may have the genotype on a patient without the phenotype and hence the pathological condition, but, in other cases, one may have the genotype with the phenotype with or without the manifestation of the pathology (i.e. the previous genetic mutation may result in a patient with the presence or absence of the prolongation of the QT segment, and, even in presence of the phenotype (long QT), one may or may not have the clinical symptoms of arrhythmias). Probably the puzzle is not yet complete, without considering the proteomic scale (see §2a).

This does not imply that a modern researcher has to have the entire spectrum of competencies from gene up to the organ (certainly, very different are the methods to be applied as well as how to design proper experimental settings), but certainly one has to possess the vision of what is the overall picture one wants to study, looking for cooperative research efforts and integrating among the different scales what could be useful for a better physiological study or also possible relevant clinical applications.

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A great effort is on course, nowadays, for creating very large databases and networking of models and technologies for integrating such information (Physiome project: Rudy 2000; Hunter et al. 2002), to be connected with genome and proteome projects and virtual physiological human project, which is inserted into the activities of the Seventh Framework Programme of the EU.

(a) The proteomic scale in the study of cardiovascular pathologies

Channelopathies are certainly a group of inherited diseases caused by specific mutations in the genes coding for Na\(^+\), Cl\(^-\), K\(^+\) and Ca\(^{2+}\) channel subunits.

Despite some success in the prediction of genetic diseases, genomics has so far failed to deliver the promised abundance of novel therapeutics. The reason is that gene structure does not provide direct information on too many important features of the proteome (the time- and cell-specific complement of the genome) and, in particular, about DNA–protein interactions, protein–protein interactions and environmental influences on protein expression and activity. Since virtually every human disease involves protein abnormalities, it is easy to understand why proteomics is beginning to cover such a central role in the post-genomic era. While shotgun sequencing has been recognized as a viable, fast and economic method for the sequencing of a whole genome, and microarrays have been demonstrated to be a fast and reliable method for globally profiling the transcriptome (i.e. the mRNA complement of the genome), a universal method for the global analysis of proteome expression seems to be far from being found yet (James 2001).

Until recently, two-dimensional polyacrylamide gel electrophoresis has been the only satisfactory approach for analysing the state of expression of the proteins in a cell. This technique allows one to separate the proteins in two complementary directions, according to their isoelectric point and to their mobility on a porous gel, which is approximately mass dependent. Two-dimensional gels, though, suffer several major drawbacks; a very limiting factor, for instance, is their inability to deal with many classes of proteins, such as hydrophobic ones and those with an isoelectric point at either extreme of the pH scale.

For this reason, in the last few years, this technology has been often enhanced or even gradually replaced by mass spectrometry, which has emerged as the tool that best fulfils all the requirements of proteomic analysis (a wide dynamic detection range, high-throughput and high-confidence protein identification and quantification, the ability to deal with multiple proteins that co-migrate in the same gel spot, and the ability to identify post-translational modifications) and allows one to identify proteins directly from genome databases.

Figure 2 shows a typical example of a proteomics workflow, which can be followed to study a variety of pathologies (from cardiovascular, to neurological, to oncological ones). Two-dimensional gels are examined to detect proteins, which present a correlate with the pathology with respect to the control group. Protein spots, which are differentially expressed, can be excised from the gel (i.e. the one circled in figure 2), digested by means of a proteolytic enzyme (usually trypsin) and analysed by means of a mass spectrometer (MS). Data analysis of the MS spectra allows one to identify peptide peaks, which can be used as a fingerprint to mine all DNA and proteins databases, in order to identify the protein under investigation (see the correspondent amino acid sequence in figure 2).
This bottom-up approach, called peptide mass fingerprinting, is based on the assumption that a protein can be univocally recognized by the recognition of few peptides. The possibility to handle peptides rather than proteins has triggered the challenge to replace gels with alternative methods of expression analysis; as an example, the coupling of two instruments (a liquid chromatograph plus a MS) is capable of directly displaying the peptides in two complementary directions, as described in Cappadona et al. (2008).

Therefore, the final issue could be that in order to find out more precise relationships between genotype, phenotype and the manifestation of the pathological event, one has to consider the correlates not only with genetic defects but also with the consequences that such a defect may have on peptide production and protein expression.

(b) Genetic influences on heart rate and blood pressure variability in monozygotic and dizygotic healthy twins

Twin studies have been a valuable source of information about the genetic basis of complex characteristic traits and diseases. Blood pressure and heart rate variability (HRV) are strongly influenced by genetic factors (Voss et al. 1996a; Busjahn et al. 1998). Genetic variability, which influences cardiovascular phenotypes, is likely to be relevant to cardiovascular diseases. Owing to the increasing impact of variability parameters in clinical diagnostics, heritability of cardiovascular regulations is of substantial interest for phenotyping of these...
cardiovascular diseases. Therefore, we tested whether HRV, blood pressure variability (BPV) and baroreflex sensitivity (BRS) are influenced by genetic factors, and therefore suited for phenotyping of cardiovascular diseases.

Short-term ECG and continuous non-invasive blood pressure were recorded in 45 twin pairs (31 monozygotic of age 33 ± 13.5 years and 14 dizygotic of age 36 ± 13 years) under resting conditions for 30 min. For analysing the beat-to-beat variability (figure 3), we calculated linear time- and frequency-domain parameters (Task Force 1996), as well as parameters from nonlinear symbolic dynamics (Voss et al. 1996b). Interactions between blood pressure and heart rate were quantified by applying a spontaneous BRS method (Malberg et al. 2002).

A genetic dependency was assumed if the parameter differences between monozygotic twins are considerably less than those between dizygotic twins (figure 3). For statistical evaluation, the group means of these differences were tested for equality between monozygotic and dizygotic twins as well as monozygotic twins and non-twins (REF) and dizygotic twins and REF using the Mann–Whitney test. REF contains the differences between one member of a twin pair and a randomly selected but age-matched member of another twin pair.

Altogether, 19 parameters from all domains showed significant differences between monozygotic and dizygotic twins reflecting a genetic background. Most significant parameters from every domain are: (i) VLF (frequency domain) and Shannon entropy (time domain) of the RR histogram from HRV (both \( p < 0.05 \)), (ii) VLF \( (p < 0.01) \) and phvar2 \( (p < 0.05, \) index of increased variability from symbolic dynamics) from systolic blood pressure, (iii) LFn \( (p < 0.05, \) frequency domain), plvar5 \( (p < 0.05, \) index of decreased variability from symbolic dynamics) and mean value \( (p < 0.01) \) of diastolic blood pressure, and (iv) number of tachycardic and bradycardic baroreflex sequences (both \( p < 0.05 \)).

In conclusion, heart rate, diastolic blood pressure, BPV and BRS are strongly influenced by genetic factors. These significant parameters can be used to characterize the influences of genetic and environmental factors on cardiovascular phenotypes, providing new insights into cardiovascular regulation and pathogenesis of cardiovascular diseases.

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Adjustment between autonomic rhythms of different time scales in chronic heart failure

The incidence of CHF is increasing in the ageing population of developed countries. Better understanding and monitoring of the entire cardiovascular functioning, which includes the readjustment of different control mechanisms with different time scales, can improve treatment and, consequently, the quality of life with a better prognosis. The most relevant clinical predictors of CHF patients' outcome are their New York Heart Association (NYHA) class, left ventricular ejection fraction (LVEF), systolic arterial pressure (SAP) and peak VO₂ at cardiopulmonary exercise testing. These factors reflect predominantly the pathophysiologically reduced performance of the heart. Compensating readjustment of autonomic and neurohumoral cardiovascular control mechanisms is necessary for maintaining the homeostasis under pathophysiological conditions (Maestri et al. 2007).

The cardiovascular control system incorporates mechanisms at different time scales: (i) vagally mediated influences with time delays to the heart of 150–550 ms capable of modulating the sinus node on a beat-to-beat basis, (ii) sympathetically mediated influences with time delays to the heart of 3 s, and several other mechanisms up to (iii) the circadian rhythm of 24 hours (Hoyer et al. 2008).

The aim of this work was to assess whether AIF-based measures reflect the complex dysfunction associated with CHF and whether a relationship exists between short- and long-term AIF, which has prognostic value.

We analysed 24-hour Holter recordings from 200 consecutive CHF patients in sinus rhythm and computed AIF indices over one heartbeat interval (BDnn) as the shortest time scale and longer intervals (PDmVLF, 12.5–166.7 s). AIF was estimated as the prediction function of Shannon entropy. Endpoint for survival analysis over 3 years (Cox model) was total cardiac death. A prognostic model was built considering known clinical and functional risk factors, and the ability of AIF indices to add prognostic information to this model.

Out of the clinical predictors, NYHA class, LVEF, peak VO₂ and SAP had the highest predictive value. When entered into a common model, both BDnm and PDmVLF added prognostic information (table 1). High risk was associated with reduced fast AIF and increased slower AIF. The Kaplan–Meier survival curves of a composite index from BDnm and PDmVLF are shown in figure 4. The same association between increased beat-to-beat complexity and decreased longer term complexity was obtained in a model simulation study as a possible mechanism of self-stabilization of a nonlinear dissipative system (Hoyer et al. 2007).

We conclude that AIF indices provide prognostic information independent of known functional and clinical risk factors in CHF patients. The readjustment between short- and long-term cardiovascular controls may provide further insights into complex dynamic coordination under pathophysiological conditions with prognostic and therapeutic implications.

3. Complex integration of multiple organs

‘Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity’ (WHO 1948). Particular conditions on somatic, mental and social level can lead to psychosomatic and psychosocial...
diseases going beyond poor somatic disturbances. However, even the somatic (systemic) function is based on the complex adjustment between several organs such as brain, heart, lungs, blood, kidney, alimentary system, immune system, etc. Hence, any isolated primary damage inevitably influences other organs of the organism.

The cardiovascular and respiratory system is designed to guarantee an adequate continuous supply of the organism with oxygen and nutrients, and an adjusted clearance of metabolic waste products. Therefore, blood flow through the body and gas exchange through the lungs are adjusted to the momentary situation by the combination of regional and higher level control mechanisms;

Table 1. Multivariate Cox prediction models; clinical variables and (a) short-term AIF, (b) long-term AIF. (Adapted from Hoyer et al. (2008).)

<table>
<thead>
<tr>
<th></th>
<th>$\chi^2$</th>
<th>$p$-value</th>
<th>HR (95% CI)</th>
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</thead>
<tbody>
<tr>
<td>(a) BDnn $\geq 0.73$</td>
<td>3.8</td>
<td>0.050</td>
<td>1.76 (1.00–3.09)</td>
</tr>
<tr>
<td>NYHA I–II versus III–IV</td>
<td>6.9</td>
<td>0.009</td>
<td>1.92 (1.18–3.13)</td>
</tr>
<tr>
<td>LVEF</td>
<td>5.6</td>
<td>0.018</td>
<td>0.95 (0.92–0.99)</td>
</tr>
<tr>
<td>SAP</td>
<td>2.4</td>
<td>0.12</td>
<td>0.98 (0.97–1.01)</td>
</tr>
<tr>
<td>peak_VO2</td>
<td>2.3</td>
<td>0.13</td>
<td>0.96 (0.91–1.01)</td>
</tr>
<tr>
<td>(b) PDmVLF $\leq 0.96$</td>
<td>4.6</td>
<td>0.031</td>
<td>1.73 (1.05–2.85)</td>
</tr>
<tr>
<td>NYHA I–II versus III–IV</td>
<td>6.7</td>
<td>0.009</td>
<td>1.90 (1.17–3.08)</td>
</tr>
<tr>
<td>LVEF</td>
<td>5.7</td>
<td>0.016</td>
<td>0.95 (0.92–0.99)</td>
</tr>
<tr>
<td>SAP</td>
<td>2.1</td>
<td>0.15</td>
<td>0.99 (0.97–1.01)</td>
</tr>
<tr>
<td>peak_VO2</td>
<td>1.3</td>
<td>0.24</td>
<td>0.97 (0.92–1.02)</td>
</tr>
</tbody>
</table>

Figure 4. Kaplan–Meier survival curves of total mortality: high-risk AIF, BDnn $\geq 0.73$ and PDmVLF $\leq 0.96$; low-risk AIF, BDnn $< 0.73$ and PDmVLF $> 0.96$. Adapted from Hoyer et al. (2008).

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those effects are closely interrelated. The functional state of the circulation and respiration is continuously monitored by receptors at various places in the cardiovascular system and the brain stem. Hence, each assessable controlled quantity such as heart rate, blood pressure or blood oxygen reflects only one element of a multi-parametric object function, and it is influenced by several other subsystems. Heart rate, blood pressure, gases and respiratory movements are those quantities. Their interrelated behaviour can be assessed, for example, by BRS, chemoreflex sensitivity and parameters of heart rate fluctuations, which reflect particular aspects of the autonomic control. The adjustment between multiple organs is essentially mediated by autonomic (neurovegetative) and humoral mechanisms.

The challenges for medical signal analysis and diagnostics are the identification of the primary pathological disturbance, the resulting secondary disturbances and the monitoring of key functions of the multiple organ (dys)function. The knowledge of these aspects, as complete as possible, is the basis for the development of beneficial therapeutic strategies and their continuously controlled application.

An example of cardiovascular dysfunction due to another systemic disease is the so-called ‘cor pulmonale’, a disease with increasing incidence in western countries. The patient suffers from symptoms such as exercise intolerance, shortness of breath, swelling of the feet (oedemas) and chest discomfort. The patient has a bluish colour of the lips (cyanosis) and an enlarged right heart. Different pathogenetic mechanisms such as pulmonary vasoconstriction, constrained pulmonary vascular bed secondary to lung disorders, increased blood viscosity secondary to blood disorders or idiopathic primary pulmonary hypertension can develop increased pulmonary arterial pressure and, subsequently, cor pulmonale. Hence, cor pulmonale is an alteration in function and structure of the right cardiac ventricle caused by a primary disorder of the respiratory system.

Cardiogenic shock is defined as an inadequate tissue perfusion resulting from pathologically decreased cardiac output, which is, for example, the case due to decompensated CHF or acute myocardial infarction. The decreased blood pressure in the organism activates several systemic compensatory mechanisms, such as sympathetically mediated increased heart rate and vasoconstriction, both additionally burdening the myocardium due to increasing oxygen demand. The systemic hypoperfusion leads to tissue hypoxia and vital organ dysfunctions. The resulting cellular hypoxia and end-organ damage can lead to multiple organ dysfunctions. The organs of vital importance are the brain, the heart and the kidneys. The diminished perfusion of the brain leads to an altered mental status ranging from confusion and agitation to flaccid coma.

Examples of complex associations of cardiovascular regulation with other organ systems will be presented concerning central nervous influences during sleep and depression, as well as concerning multiple organ diseases in ICU.

(a) Depression changes autonomic control

Today, it is known by clinical studies that depressive disease is associated with increased risk for cardiovascular morbidity and mortality. A possible explanation is that depression influences the autonomic nervous system increasing sympathetic
and/or reducing parasympathetic modulation. To evaluate this hypothesis, several studies have been performed (Voss et al. 2006a; Boettger et al. 2008). While definite mechanisms for this cardiac vulnerability are unknown, it is assumed that an altered autonomic neurocardiac regulation is at least one important pathophysiological factor. However, at the moment, it is quite unclear whether an autonomic dysregulation plays a major role in this manner.

In this study, two populations were enrolled: 25 patients suffering from depression who had not taken antidepressants for at least eight weeks prior to the investigations (untreated depressed patients, UDP) and 72 matched healthy controls (CON); the patients fulfilled the ICD-10 criteria for depressive episodes. The severity of depression was assessed based on a semi-structured clinical interview, the Beck’s depression inventory and Hamilton depression rating scale.

From the 30 min data records (ECG, non-invasive blood pressure), time series of heart rate (tachograms) consisting of successive beat-to-beat intervals (BBI), systograms (systolic blood pressure values over time) and diastograms (diastolic blood pressure values over time) were extracted and several parameters of the time and frequency domains and nonlinear dynamics were calculated.

Comparing UDP with CON parameters from time domain (table 2), BRS and symbolic dynamics but not the index of the so-called sympathetic vagal tone (LF/HF) showed univariate significant differences ($p<0.05$).

The obtained results show that acutely depressed patients who had not taken antidepressants differ significantly in linear (Task Force 1996) and nonlinear parameters from heart rate and BPV, suggesting a considerable dysfunction of the autonomic regulation. In addition, the nonlinear indices phvar10 from symbolic dynamics (Voss et al. 1996b) and compression entropy (CE; Baumert et al. 2004) clearly show a decreased complexity of heart rate regulation in depressed patients. However, no differences within high- (HF) or low-frequency (LF) components and the ratio LF/HF suggesting a vagal withdrawn were

<table>
<thead>
<tr>
<th>parameter</th>
<th>$p$-value</th>
<th>CON</th>
<th>UDP</th>
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</thead>
<tbody>
<tr>
<td>meanNN</td>
<td>$&lt;0.05$</td>
<td>930.8 ± 139.8</td>
<td>855.3 ± 157.9</td>
</tr>
<tr>
<td>sdNN</td>
<td>$&lt;0.05$</td>
<td>63.9 ± 22.1</td>
<td>50.6 ± 21.4</td>
</tr>
<tr>
<td>rmssd</td>
<td>$&lt;0.01$</td>
<td>49.4 ± 22.9</td>
<td>35.1 ± 19.2</td>
</tr>
<tr>
<td>pNN50</td>
<td>$&lt;0.01$</td>
<td>0.27 ± 0.19</td>
<td>0.16 ± 0.17</td>
</tr>
<tr>
<td>LF/HF</td>
<td>n.s.</td>
<td>2.60 ± 2.55</td>
<td>2.43 ± 1.71</td>
</tr>
<tr>
<td>phvar10</td>
<td>$&lt;0.01$</td>
<td>0.32 ± 0.17</td>
<td>0.21 ± 0.17</td>
</tr>
<tr>
<td>CE</td>
<td>$&lt;0.01$</td>
<td>0.74 ± 0.08</td>
<td>0.66 ± 0.11</td>
</tr>
<tr>
<td>sys_meanNN</td>
<td>n.s.</td>
<td>121.2 ± 13.74</td>
<td>127.8 ± 22.0</td>
</tr>
<tr>
<td>dia_meanNN</td>
<td>$&lt;0.05$</td>
<td>76.4 ± 10.4</td>
<td>83.3 ± 14.6</td>
</tr>
<tr>
<td>bslope</td>
<td>$&lt;0.01$</td>
<td>19.4 ± 6.5</td>
<td>14.0 ± 6.3</td>
</tr>
<tr>
<td>tslope</td>
<td>$&lt;0.01$</td>
<td>18.8 ± 6.5</td>
<td>14.4 ± 6.2</td>
</tr>
</tbody>
</table>

Table 2. Univariate significances for discrimination between untreated depressed patients (UDP) and health subjects (CON). (meanNN, sdNN, rmssd, pNN50, standard parameters from HRV time-domain analysis; LF/HF, standard parameter from HRV frequency-domain analysis; sys_meanNN, dia_meanNN, systolic, diastolic mean blood pressure; plvar10, parameter from symbolic dynamics; CE, compression entropy; bslope, tslope, parameters from BRS.)
obtained. This is partly in contrast to other studies (Guinjoan et al. 1995), which have shown in depressed patients a diminished parasympathetic reactivity, and presumably an increased sympathetic reactivity.

We could demonstrate that patterns of heart rate regulation in depressed patients differ in nearly all domains but not in the homeostasis physiological balance (interplay between the sympathetic and parasympathetic nervous system) in comparison with control subjects.

(b) Sleep intended as a multiorgan manifestation

As is well known from the analysis of polysomnographic recordings, sleep is clearly the manifestation of a multiorgan involvement; among these are central nervous, cardiovascular, respiratory, muscular, endocrine–metabolic systems and so on (Bianchi et al. 2006). The study of the effect of a simple arousal during sleep in normal or a pathological condition is a good example of such multiorgan synchronism. In fact, this physiological episode involves the interaction of different organs in the internal homeostasis maintenance. An arousal is defined as an abrupt shift in EEG frequency with respect to the background frequency. An arousal has different roles during sleep. First, the arousal acts as radar, which activates specific systems to detect possible risks in the environment. Second, the arousal helps in the construction of the sleep macrostructure. Finally, arousal activates specific systems in order to overcome pathological internal situations as is an apnoea episode (Narkiewicz et al. 1999). Obstructive sleep apnoea (OSA) consists of an interruption in the airflow to the lungs caused by an occlusion in the upper airways. Sleep apnoea produces reductions in the blood oxygen saturation and arousal events. During OSA, respiratory muscles produce mechanical efforts in order to overcome the occlusion. If these efforts are not sufficient, the oxygen in the blood begins to decrease, muscle efforts increase as a response to the hypoxia, until an arousal takes place to reactivate all the systems and restore the respiration. Figure 5a(i)–(vi) shows electroencephalogram (EEG), electromyogram (EMG), ECG, airflow (FL) via nasal thermistor, abdominal effort (ABD) and oxygen saturation (SaO2) during OSA in non-rapid eye movement sleep. One can observe that, in association with the arousal episode, there are activation of the muscles, increase in the heart rate, breath restore and, as a consequence, an increase in the oxygen saturation. Note how an external event produces the interaction at the multiorgan level to maintain the level of the physiological parameters and to ensure a physiological condition. Figure 5b shows the behaviour of the heart rate variations during an apnoea episode. We can observe how the spectral components of the heart rate variations are correlated with the episode. During the episode, a frequency component around 0.04 Hz appears, thus indicating an increase in the sympathetic drive (Mendez et al. 2008).

(c) Risk assessment in multiple organ dysfunction syndrome

Multiple organ dysfunction syndrome (MODS) is a multiple systems illness, representing a common end-stage pathway of inflammation, infection, dysfunctional host response and organ failure in critically ill patients, frequently leading to death (Marshall 2000; Seely & Christou 2000; Seely & Macklem 2004). A MODS is a sequential failure of several organ systems after a trigger event, such as cardiogenic shock or decompensated CHF, whereby the associated autonomic dysfunction may substantially contribute to the development of...
MODS. The clinician’s question is whether HRV indices characterize the complex systemic dysfunction additionally to the numerous clinical parameters leading to better risk stratification and clinical treatment.

Figure 5. Typical example of a polysomnography record during OSA: note the multiorgan participation during an arousal episode. (a) (i) Electroencephalogram (EEG), (ii) electromyogram (EMG), (iii) ECG, (iv) airflow (Fl), (v) abdominal effort (ABD) and (vi) oxygen saturation (SaO₂). (b) (i) RR intervals obtained from the ECG signal and (ii) its time–frequency representation (via short-time Fourier transform, Born–Jordan kernel).

Phil. Trans. R. Soc. A (2009)
Complexity and coupling are two outstanding aspects of multiple organ functioning. Decomplexification in critical illness (Goldstein et al. 1998) and uncoupling as essential systemic dysfunction in MODS (Godin & Buchman 1996) seem to open a new window into understanding pathogenesis and hence developing new strategies of treatment (Hoyer et al. 2006). Godin & Buchman (1996) proposed a concept of ‘uncoupling’ by altered neurally mediated organ interactions in MODS and sepsis. An intact ‘anti-inflammatory cholinergic reflex’ (Borovikova et al. 2000; Tracey 2002; Libert 2003), which might be reflected by respective heart rate rhythms, seems to be a precondition to suppress the overwhelming inflammatory response in MODS and sepsis. The altered AIF found in MODS patients (Hoyer et al. 2006) supports the concept of systemic decomplexification. The reduced VLF (power in the very-LF band of heart rate fluctuations) can be interpreted by the alteration of the cooperation of the various underlying mechanisms and hence as an indicator of vagal activity (Schmidt et al. 2005). Taylor et al. (1998) demonstrated in young healthy people that VLF is mainly associated with parasympathetic outflow. Saul et al. (1991) demonstrated that the parasympathetic system mediates heart rate fluctuations in all frequencies. Consequently, VLF may be a key factor to assess anti-inflammatory cholinergic activity, which essentially influences the prognosis of the complex systemic processes.

We investigated the hypothesis that VLF provides additional prognostic information to the established clinical APACHE II score in MODS patients.

The study was designed as a follow-up over 60 days. A cohort of 78 MODS patients (40 endpoints) with stable sinus rhythm was included. In these subjects from 24-hour Holter recordings, clean (less than 5% corrected ectopies or artefacts) datasets over at least half of the night-time (00.00–05.00) and half of the daytime (09.00–19.00) were analysed by the established HRV measures (Task Force 1996). Independent prognostic value of APACHE II score and HRV indices (both quantized for quintiles) was assessed by multivariate Cox proportional regression models using stepwise backward parameter elimination. The prognostic value of the resulting model was demonstrated by Kaplan–Meier survival curves.

As a result, higher mortality was associated with increased APACHE II score and decreased VLF. Both APACHE II score and VLF significantly predicted the 60 days mortality in the univariate analysis. The multivariable prognostic model demonstrates independent significant contribution of APACHE II score and VLF (the best predicting HRV index; table 3), however, without improving the overall hazard ratio. The resulting Kaplan–Meier survival curves are shown in figure 6.

In conclusion, the Cox proportional regression models of the example presented show that VLF provides independent prognostic value to the clinically established APACHE II score. The high prognostic value of the univariate VLF Cox model accentuates the potential ability of VLF as a substitute of APACHE II score and hence a way of continuously assessing systemic risk based on HRV indices.

We hypothesize that analysis of variability and connectivity of individual variables offers a novel means of evaluating and differentiating the systemic properties of a complex nonlinear system, along the research line suggested by Seely & Christou (2000). In that connection, HRV indices such as VLF in the present study were found to play an important role.
4. Multivariate data analysis

The definition of multivariate analysis refers to statistical techniques used to analyse data that arise from more than one variable. This essentially models biomedical engineering reality where each situation, diagnosis, therapy or any other decision involves more than a single variable.

Most physiological and pathophysiological behaviours include an interaction of various single processes. Therefore, we have to expect that parameters or measures with a different origin have to be considered in a multivariate way independently if they are derived from one process, one organ or from multiple processes and organs.

Statistical multivariate analysis methods are especially:

— cluster analysis;
— factor analysis (e.g. principal component analysis);
— multidimensional scaling;
— correspondence analysis;

Table 3. Cox proportional regression models.

<table>
<thead>
<tr>
<th></th>
<th>p-value</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>univariate models</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APACHE II</td>
<td>0.008</td>
<td>1.36 (1.08–1.70)</td>
</tr>
<tr>
<td>VLF</td>
<td>0.008</td>
<td>0.72 (0.57–0.92)</td>
</tr>
<tr>
<td><strong>multivariate models</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APACHE II</td>
<td>0.060</td>
<td>1.25 (1.00–1.60)</td>
</tr>
<tr>
<td>VLF</td>
<td>0.034</td>
<td>0.97 (0.56–0.98)</td>
</tr>
</tbody>
</table>

Figure 6. Kaplan–Meier survival curves for 60 days mortality in MODS patients using a bivariate model of APACHE II score and VLF.
— classification methods (e.g. discriminant analysis, support vector machines);
— canonical correlation analysis;
— ROC curve (receiver operating characteristic);
— regression models (e.g. logistic regression, Cox regression; nonlinear model: neural networks); and
— multivariate analysis of variance (MANOVA).

Data from the cardiovascular system are partly easy to collect; what we really need in complex problem solving is information. As in the measurement process itself, appropriate instruments of reasoning must be applied to the data interpretation task. Effective tools serve in two capacities: to summarize the data and to assist in interpretation. The objectives of interpretive aids are to reveal the data at several levels of detail. This leads to the need of combining single information from subsystems developing the highest degree of global information for the requested special task.

The interest in multivariate analyzing and interpreting cardiovascular oscillations as signs of physiological or pathophysiological cardiovascular regulation processes has increased since the introduction of non-invasive HRV analysis in 1987. While investigating at the beginning (e.g. Cripps et al. 1991; Bigger et al. 1992) only linear measures of variability, later on nonlinear methods of HRV analysis (e.g. Cerutti et al. 1996; Voss et al. 1996b) and multidimensional analysis including respiration and blood pressure signals have been increasingly considered (e.g. Parati et al. 1995; Bianchi et al. 2006).

The following examples demonstrate the ability of multivariate analysis in cardiovascular research combining modern non-invasive and, especially, nonlinear methods of variability analysis with established clinical parameters.

(a) **Risk stratification in heart failure patients**

Approximately 50 per cent of patients with CHF suffer from cardiac death within 4 years. The aim of this study was to develop a multivariate parameter set for an enhanced risk stratification in patients with CHF using ECG, heart sound (HS), HRV and BPV analysis methods.

ECG and continuously non-invasive blood pressure were measured for 30 min and, in addition, the HS (via electronic stethoscope) over five heartbeats on nine auscultation areas in 43 CHF patients characterized by a NYHA value greater than or equal to 2. Parameters that describe the first and second HS were calculated using a wavelet-based HS analysis method (Voss et al. 2005). For the analysis of beat-to-beat variability, linear time and frequency domain (Task force 1996) and indices from nonlinear dynamics (Voss et al. 1996b; Baumert et al. 2002) were calculated. Additionally, the ECG and blood pressure morphology (BPM; Voss et al. 2006b) were investigated. After a six-month follow-up, the CHF group was divided into two subgroups: CHF with no progression of the disease (CHFlow, n=21, low-risk group) and with significant progression of the disease including seven patients who suffered from cardiac death (CHFhigh, n=22, high-risk group).

The Mann–Whitney U-test (p < 0.05) was assessed for statistical evaluation. Multivariate parameter sets were estimated (table 4) by stepwise discriminant function analysis with cross-validation separately for clinical parameters, HRV indices, HS/ECG indices and, finally, for all parameters.

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Figure 7 shows the ROC curves of the parameter sets. There is a remarkable increase in discriminant power integrating single parameters into a multi-parametric set (ejection fraction and NYHA versus clinical set; HS1 as the best univariate parameter from all domains versus HS and ECG set). Furthermore, comparing all parameter sets, it is obvious that the final set differentiates best between the two groups.

For risk stratification in differentiating the groups CHFhigh and CHFlow, an optimal set of three univariate significant parameters was determined and achieved a cross-validated sensitivity of 91.7 per cent, a specificity of 80.0 per cent and an accuracy (area under ROC) of 85.2 per cent. This parameter set consists of linear and nonlinear parameters from HS, joint symbolic dynamics (interactions between blood pressure and heart rate) and BPM.

(b) Assessment of cardiovascular disorders and survival

In clinical studies, multiple variables and confounders have to be considered. Resulting sample sizes (number of patients) depend on: (i) the effect, which means the relevance of the quantity difference, (ii) the type I error (false positive, wrongly rejecting a null hypothesis), (iii) the type II error (false negative, wrongly accepting a null hypothesis), (iv) the data characteristics, and (v) their vice versa dependencies (Fletscher & Fletcher 2005). The sample size design also depends on the context and the objective of the study. Particular selected questions of pathophysiological interest can be investigated in small, well-defined study cohorts. However, in larger populations suffering from the same disease, the number of clinical confounders is large and has to be considered by a respective sample size design, appropriately selected and abstracted key factors, and statistical validation methods.

Some of those aspects of multivariate analysis are demonstrated by two clinical studies as follows.

First, an investigation of dependencies between heart rate and respiratory movements in patients after myocardial infarction will be discussed. A cohort of 39 patients with LVEF of 60±17 per cent, left ventricular end-diastolic diameter (LVEDD) of 52±5 mm and median age of 62 years was compared with a reference group of 24 subjects (median age 53 years). The cardiorespiratory
measurements were taken in supine position over 10 min at a sampling rate of 1000 Hz. From the heartbeat interval series and respiratory movements (calculated from ECG amplitudes), indices of (i) HRV according to Task Force (1996), (ii) cardiorespiratory cross-correlation (CC) and (iii) cardiorespiratory phase synchronization (Hoyer et al. 2002) were analysed.

The multivariate discriminatory power was based on significant measures selected by backward stepwise logistic regression.

The HRV indices significantly discriminated the patients from the normal group AUC (95% confidence interval) = 0.80 (0.70, 0.91). Both final multivariate models, using [HRV + cardiorespiratory CC] with AUC = 0.88 (0.80, 0.96) and [HRV + cardiorespiratory phase synchronization] with AUC = 0.84 (0.73, 0.94) improved the diagnostic accuracy.

It can be concluded that HRV and cardiorespiratory interdependencies provide additional independent diagnostic value and should be considered in the design of respective prognostic studies. In such a survival study, candidates for risk factors could be an HRV index, a cardiorespiratory coupling index, NYHA class, LVED, LVEDD, beta-blocker, diabetes mellitus, etc. Besides the number

Figure 7. ROC curves from (a) clinical parameter set (not cross-validated; dotted line, NYHA; dashed line, ejection fraction), (b) HRV parameter set, (c) HS and ECG parameter set and (d) the final, optimal parameter set (dotted line, best univariate parameter HS1 from HS analysis). Each set consists of three parameters automatically selected by stepwise discriminant function analysis.
of risk factors, the resulting sample size design is essentially determined by the mortality of approximately 5 per cent in post MI patients (references from Kleiger et al. 2005).

A study cohort of 200 patients (97 endpoints) consecutively admitted to a specialized CHF unit for evaluation and treatment in conjunction with heart transplantation provided an appropriate sample size for a survival analysis over 3 years using the following set-up. Essential clinical, functional and HRV variables were pre-selected: aetiology (ischaemic versus non-ischaemic); NYHA class; LVEF; LVEDD; resting heart rate; sodium; age; SAP; peak VO₂ at cardiopulmonary exercise; SDNN; AIFshort; and AIFlong. Furthermore, these variables were abstracted by dichotomization. The non-predicting variables were eliminated by backward stepwise Cox regression models. The final models were validated by bootstrap analysis. The final results are presented in table 1.

From the traditional HRV indices, only SDNN provided prognostic value additional to the clinical and functional parameters. Both AIF indices removed SDNN from the multivariate Cox regression models. A composite index AIFshort (BDnn) and AIFlong (PDmVLF), furthermore, improved the prognostic value of heart rate pattern (figure 5) and might be an appropriate abstracted HRV index in the context of clinical risk assessment in these patients (for details see Maestri et al. 2007; Hoyer et al. 2008).

(c) Atrial fibrillation

Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice, constituting a major risk factor for stroke and mortality from cardiovascular and other causes. AF is a supraventricular arrhythmia, characterized by desynchronization of atrial electrical activity. According to the multiple wavelets hypothesis (Moe 1962), AF is characterized by circling waves propagating randomly throughout the atria generating complex and ever-changing patterns of electrical activity in which waves are fragmented or mutually annihilated. However, the presence of circling waves tends to synchronize atrial regions excited by the same wavefront and the emergence of spatio-temporal organization may be observed (Jalife et al. 1998). The extent of this organization is supposed to be related to the number of circulating wavelets, the probability of self-termination of AF episodes and the energy required for cardioversion, and it could guide appropriate antiarrhythmic therapy.

The concept of organization may be investigated from the mutual analysis of pairs of recordings simultaneously collected, thus judging the electrical activity at one site in relation to the activity at the second one.

Three methods for the analysis of the relationships between atrial activity recorded during normal sinus rhythm and AF have been compared (Mainardi et al. 2006). They included (i) a CC-based index, (ii) a NLA index and (iii) the synchronization (S) index.

From a methodological point of view, the three metrics provide information and quantify the coupling between signals from different perspectives. The CC index accounts for the second-order moment of the signals, while it neglects the information contained in higher order moments or the nonlinear mechanisms. Conversely, these mechanisms are considered by both NLA and S. In particular, NLA expresses the reduction of variance of x obtained, predicting the x values on
the basis of $y$: the better the prediction, the more minor the unexplained variance and the more major the NLA index. Index S is, instead, derived from normalized cross-conditional entropy (see also Porta et al. 2001) and appears to be sensitive to various signal coupling mechanisms (linear or not) including $1:N$ sub-harmonic links between signals, with superior performance with respect to CC evidenced in many biosignal applications. A comparative analysis of NLA and S gives evidence that, in NLA estimate, only conditional probabilities between samples are computed. Conversely, in the definition of S, the conditional probabilities of patterns are also involved. Therefore, S results are less sensitive to spurious coupling mechanisms and should be more reliable when the existence of coupling has to be excluded.

In summary, one can state that, for complex analyses of cardiovascular oscillations, a multivariate approach is essential. This multivariate approach leads in most cases to a reduced number of relevant parameters, reveals optimal parameter sets and, therefore, leads to a maximum of available information about the investigated system.

5. Concluding remarks

Advanced approaches in the study of the cardiovascular system allow one to explain various complex phenomena in its regulation processes. Modelling of these physiological behaviours takes benefit from multivariate analysis, i.e. considering more realizations from the same system. Furthermore, important physiological and clinical findings can be obtained from the interaction among different physiological systems (i.e. central and autonomic nervous system, respiratory system, endocrine–metabolic system, etc.), thus fulfilling a multi-organ approach. Finally, it has been demonstrated how the integration among different spatial scales (from genes up to the whole organ), and temporal scale on the same signals could bring new fundamental and solid support to recover the lacking information, which might contribute to give an answer to the following question: why does the same genotypic pattern not necessarily bring the same phenotypic pattern and hence the manifestation of the pathological event?

Therefore, the issue of MMM paradigm (multivariate, multiorgan and multiscale) seems promising for a real assessment of cardiovascular complex phenomena.

Innovative applications of these techniques are expected in risk stratification of cardiovascular pathologies, for a better diagnosis of several cardiac pathologies as well as in the assessment of their pathophysiological correlates.

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