The Paradigm of Systems Biology Applied to Cardiovascular Diseases

Evandro Tinoco Mesquita¹, Eduardo Nani Silva¹, Antonio José Lagoeiro Jorge¹, Bruna de Melo Mariano¹, João Paulo Pedroza Cassino¹, Celso Vale Souza Junior¹, Michelle Araujo Mesquita², Ruíza Gonçalves Rocha¹

¹Universidade Federal Fluminense - Departamento de Clínica Médica - Niterói, RJ - Brazil
²Universidade do Estado do Rio de Janeiro - Departamento de Clínica Médica - Rio de Janeiro, RJ - Brazil

Abstract

Based on individual analyses of the functional components of an organism, the Oslerian method shows signs of depletion when attempting to explain the pathophysiology of complex syndromes such as cancer and cardiovascular diseases. This is why it is gradually being supplanted by a new paradigm: the methodology of biological systems. This new model strives to integrate knowledge in different modern research areas with the omics sciences and bioinformatics, in order to develop biological networks leading to a better understanding of these complex syndromes. The purpose of this review is to introduce clinical cardiologists and cardiovascular researchers a new tool called systems biology, showing how it integrates data from the omics sciences and its contribution to a new approach to cardiovascular disease. To date, a search of the Medline database has been conducted with the following key words in Portuguese and English: “biologia de sistemas”, “insuficiência cardíaca”, “síndrome metabólica” e “arritmias cardíacas”; “systems biology”, “heart failure”, “metabolic syndrome” and “cardiac arrhythmias”. This led to the conclusion that systems biology must be used to an increasing extent for a better understanding of complex cardiovascular diseases such as metabolic syndrome, atherosclerosis, hypertension, heart failure and cardiac arrhythmias. Cardiologists, cardiovascular researchers, other healthcare practitioners and basic researchers in other fields of knowledge will build up closer links in a quest to identify health and disease network models that are now called network medicine.

Keywords: Systems biology; Cardiovascular diseases; Heart failure

Introduction

The conventional model broadly employed in medicine is based on a reductionist view, which hinders an integrational analysis of the health and disease processes. The impossibility of solving a growing number of problems led to a crisis of model, paving the way to a new paradigm. The methodology of biological systems arises as a replacement for the reductionist model. This reality is already observed in oncology and is at an early stage in cardiology¹².

This new view seeks to integrate a series of new knowledge emerging from the omic sciences and bioinformatics to build network-organized models — the network medicine — evidencing a dynamic interaction among different elements existing in the health-disease context and between them and environmental, social, psychological and biological factors, thus broadening their understanding¹³.

Over the last two decades, there was a technical and scientific development in the areas of omic
sciences (epigenomics, genomics, proteomics, transcriptomics and metabolomics), which yielded a great amount of information about abnormalities existing in cells, tissues and organs in the context of health and cardiovascular diseases. Besides this, there was an improvement of the capacity to store and process epidemiological data, along with those coming from molecular biology tests.\(^6\)

The developments of omic sciences leveraged the approach to biological system, allowing a better understanding of the disease mechanisms, redefinition of their different phenotypes and the development of a new taxonomy, which altogether will allowing spotting new therapeutical targets.\(^5\)

The objective of this review is to introduce to clinical cardiologists and cardiovascular researchers a new tool referred to as systems biology and the manner how it integrates data from omic sciences, in addition to its contribution to a new approach to cardiovascular diseases.

**Systems Biology: an new perception about health and disease**

The Oslerian model, still predominant in the medical practice, identifies the presence of a given disease through the anatomical abnormalities existing in organs and tissues. This model, which has been improved over the last 100 years, has a reductionist approach, focusing the individual analysis of the functional components of an organism, on the belief that this sort of methodology would allow obtaining an effective explanation about how it is organized and how its functions are kept in health or disease. This way, a number of components is identified, which are subsequently correlated to existing clinical phenotypes.\(^6\)

In the cardiology area, the paradigm of cardiovascular continuum has been used to demonstrate the gradual evolution by the presence of risk factors, which lead to inflammatory changes to the vascular tissue, development of the atherothrombotic process, onset of the clinical phenotype of acute myocardial infarction, followed by structural and functional changes of the left ventricle (cardiac remodeling) that are associated with sudden cardiac death and heart failure (HF) (Figure 1).\(^7\)

This strategy allowed considerable developments from the standpoint of cardiovascular disease treatment and prevention, although, over the last decades, there has been an increasing number of information from the omic sciences and cardiovascular imaging that points to the need for broadening this perception and incorporating the concepts of network medicine.\(^8\)

Although the isolated change in a single gene has relevance to impact the pathogenesis, the clinical manifestations of a cardiovascular disease are rarely the result of abnormalities in only one effector. In fact, they most frequently are the integrated result of multiple pathophysiological routes that interface through an intricate network. The proposal to try to understand this network found in the composition of a healthy organism and the pathological conditions that impact it is richly exploited in this field, and its use is increasing over the last decades: the systems biology, an inextricable part of network medicine.\(^1,9,10\)

This new paradigm about the organic components is not a recent development in the scientific community, and its employment in medicine dates back before the molecular biology revolution. Nevertheless, in the pre-genomic era, its effectiveness was plausibly compromised by the absence of the technologies necessary for reaching this integration and investigating these systems in-depth.\(^4,10\)

The scientific developments in the areas of molecular biology, bioinformatics and the development of new technologies with an enhanced data processing at lower costs facilitated the collection of more comprehensive information, despite more capable of subsidizing more detailed approaches. On account of this, it explains the biological phenomena not only by detailing each functional component, but also through the building of networks that involve the interactions between them, unveiling formerly-unknown properties of the system studied.\(^8\)

The main foundation underling the systems biology is that “the whole is bigger than the sum of the parts involved,” i.e., the complex system presents intrinsic properties that may not be directly derived from the addition of individual structures.\(^9\)

**ABBREVIATIONS AND ACRONYMS**

- AF – atrial fibrillation
- HF – heart failure
- miRNAs – MicroRNAs
- MS – Metabolic Syndrome
- TMAO – Trimethylamine N-oxide
- TTR – transtirretin
- VSMC – regulate vascular smooth muscle cells
Figure 1
Reductionist model of cardiovascular continuum
Source: adapted from Dzau and Braunwald

This paradigm allows a new understanding of the concept of disease, which may be defined as the cessation of the cooperation between some biological parts in an organic system, leading to the jeopardy of the entire organism’s function. The said interferences may fall under one of the several network hierarchical levels, from its intracellular structure to the tissue level, in addition to that existing between several organic systems. Disturbances of one factor of the biological network can trigger a cascade effect that affects the system function as a whole (Figure 2).^9,11^.

The work with networks is responsible for simplifying complex systems, allowing a structure in which its formative and functional components interact in a self-organizing biological network. Through computer programs a network scheme can be designed with interconnections referred to as nodes, edges and hubs, which, when integrated, form functional groups referred to as modules within a complex network (Figure 3). These networks are scale-free and this architecture bestows an evolutionary advantage, given that there is a multiplicity of alternative routes to reach one node or another; and this “redundancy” also allows that the networks adapt more easily to environment changes.^1^.

At each level, the network obtains new properties not foreseen by the previous levels, demonstrating the concept of the emerging property. These networks, more than the very components, are the elements determining the physiological behavior of the organism, and, once changed, participate in the disease process.^1,4^.

The module associated with a given disease represents a group of network components that altogether contribute to the cellular function and, when dysfunctional, result in a given pathological phenotype. Each disease has its own module, though the possibility of overlapping between different phenotypes is not discarded. The onset of a disease is, therefore, seen as a combinatory problem in which multiple effects and disturbances result in a similar phenotype, considering that they change the module’s activity.^9^.

An important step toward the approach of systems biology is the spotting of this disease module for the purpose of defining the pathological phenotype, allowing the quest for new genes and better therapeutical targets for developing new drugs. Another important application of this approach is the discovery of new biomarkers targeted at monitoring the functional integrity of networks when disturbed by diseases, in addition to allowing a better capacity for classifying them.^9^.
In 1988, Gerald Reaven reported that the insulin resistance and, consequently, the increase of blood levels, were connected to a number of metabolic disorders often observed in obese individuals. He also noticed that this phenomenon, which he initially referred to as “X Syndrome,” was directly connected to cardiovascular problems and Type 2 diabetes\textsuperscript{12}. Currently, this condition is referred to as Metabolic Syndrome (MS) and defined as a set of risk factors that, when observed in one individual, exponentially increase the chances of development of many diseases and syndromes, such as cardiovascular diseases, cancer, cirrhosis, kidney diseases, among others. The metabolic syndrome, which presents multiple definitions, may be characterized by the existence of at least three of the following factors: increase of waist circumference.
circumference, blood pressure, dyslipidemia (increase of triglycerides and reduction of HDL) and hyperglycemia^{13-15}.

One of the most relevant clinical aspects in MS is the central obesity and, therefore, its pathophysiology has been broadly studied integrating different aspects, such as: low intensity inflammation, coagulation disorder/thrombosis and neurohumoral abnormalities triggered by the changes of phenotype of different cells existing in the adipose tissue. An important finding is the exacerbated adipocyte multiplication, generating the non-regulation of the mechanism of adipokine secretion, bringing on the increase of leptin, resistin and free fatty acids. Besides this, chronic inflammation is induced due to high levels of mediators such as TNF-α and IL-6, from adipocytes and macrophages^{15,16}.

The role played by these macrophages in the adipose tissue in MS has been progressively elucidated, being an aging adipocyte, a class of cells that, at a given time, cease to divide and start to produce a high load of inflammatory cytokines. This leads to the intensification of the inflammatory process in the adipose tissue, thus magnifying the mechanism of secretion of cytokines and all the consequences^{17}. With this, the forefront role of adipocytes in the pathophysiology of the metabolic syndrome is evident. These phenomena act jointly by changing the metabolism of several organs, like liver, pancreas, muscles, hypothalamus and, mainly, the cardiovascular system.

The genetic component of MS has been pointed out more recently and has spotted genes and their impact on certain metabolic routes. The new strategies, based on the systems biology, which are capable of integrating information about several genes simultaneously achieved an important role in casting light on unknown aspects of this syndrome^{18}.

The function of the bacterial microbiota existing in human mucosa in health and disease processes has been progressively elucidated, so that new evidence point to its role in the onset of obesity and metabolic syndrome^{18}. Recently the consumption of red meat has been spotted as a risk factor for the development of coronary atherosclerosis. There is a complex interaction between the carnitine, present in this type of meat, and the gut microbiota, leading to the formation of a substance referred to as Trimethylamine N-oxide (TMAO), which, absorbed into the blood, presents inflammatory properties and is supposed to be connected to the development and/or progression of atherosclerosis^{19}(Figure 4)^{20}.

The obesity is currently a public health concern, given that a significant part of the population is in the overweight range or above the reference values. Based on the concept of network medicine, the vision of social network recently reported for obesity points to the direct influence in the chances of a person to gain weight, so that when someone close, such as a friend or relative is obese, the number of other obese people in the same social network increases (Figure 5)^{12}.

A number of cardiometabolic conditions — dyslipidemia, obesity and diabetes —, as well as complications — peripheral vascular disease and acute coronary syndromes — are currently subject to clinical trials for new therapeutic approaches based on the
knowledge extended by the systems biology, including the line of MicroRNAs (miRNAs)\textsuperscript{20-22}. MicroRNAs are non-coding RNAs having approximately 22 nucleotides that link the targeted RNA sequences, interfering with the expression of a gene. Current studies reveal that these molecules work as key mediators in several physiological and pathophysiological processes that involve the vascular biology and the lipid metabolism.

Within the context of vascular diseases, there is a complex network in which miRNAs are capable of regulating the function of endothelial cells that, when changed, may trigger inflammation, vasoreactivity and angiogenesis, contributing to the onset and progression of hypertension, thrombosis and atherosclerosis. One of the networks involve miR-126, which controls the vascular inflammation, as well as regulates the angiogenic signaling and the vascular integrity\textsuperscript{23}. Additionally, plasma-derived vesicles containing this miRNA have their expression significantly reduced in patients with Type 2 Diabetes. In turn, miR-210 plays a capital role in the response to hypoxia, which occurs during cardiovascular events, such as myocardial infarction, promoting angiogenesis\textsuperscript{24}.

MicroRNAs are also important to regulate vascular smooth muscle cells (VSMC), which trigger the secretion of collagen and elastin, which are important for the muscle tone of major arteries. The microRNA has the function of controlling VSMC and, this way, interfere with the process of restenosis and progression of vascular diseases, such as atherosclerosis and abdominal aortic aneurysm.

Other area under study is the lipid metabolism and the process of atherosclerosis. Recent studies reveal that miRNAs are potential targets for new therapies, since they are involved in the regulation of lipid homeostasis. The miR-122 is increased in patients with hyperlipidemia, so that its silencing helped increase hepatic $\beta$-oxidation and decrease the synthesis of cholesterol and triglycerides. In addition to this, there are other miRNAs which participate in the regulation of lipogenesis, and are still studied as possible therapeutic targets within the context of coronary artery disease, peripheral vascular disease and obesity\textsuperscript{21}.

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**Figure 5**
Interaction between networks: metabolic, disease and social networks
DM - diabetes mellitus; CAD - coronary artery disease.
Source: adapted from Barabási\textsuperscript{12}
Cardiac arrhythmias

The last generation sequencing, models based on stem cells recreating cardiomyocytes and genetically-modified rat models are contributing to the generation of a robust database and the best understanding of molecule mechanisms in the onset of arrhythmias. The integration of information of this database with information coming from populational omic science may help develop the new area of application of the systems biology in the studies of cardiac arrhythmias.

Conditions such as atrial fibrillation (AF) and sudden death associated with channelopathies represent two areas where the depletion of the reductionist model for the development of therapeutical alternatives can be noticed. Few drugs are currently available and are considered safe to treat arrhythmias, and there is a reduced number of new drugs approved for the clinical use over the last two decades. Besides this, new approaches using invasive and high-risk techniques have been incorporated to the clinical practice, such as: radiofrequency ablation for atrial fibrillation and implantable automatic cardioverter defibrillator for primary and secondary prevention of sudden death.

The AF, the most prevalent sustained cardiac arrhythmia in clinical practice, is an example in which the clinical heterogeneity has hampered the genetics-based research studies, which renders it capital to develop experimental models in association with the populational study for the better understanding of this condition amongst the elderly people, obese people, diabetics, as well as given ethnical groups and geographical locations.

This arrhythmia has also been associated with a number of structural left atrium abnormalities: chamber dilation, fibrosis, amyloid deposition and, more recently, inflammation, ischemic phenomena, increase of intra-atrial pressure and abnormalities associated with neuroanatomical system factors. Besides this, genetic markers have been developed based on studies with families of individuals with AF.

An initial approach starts to be defined aiming to identify individuals with higher susceptibility to AF and, with it, integrating the experimental and populational knowledge to develop drugs for a given point of the biological network, working with lower risk of proarrhythmia for patients.

Several genes have already been spotted to be associated with AF. The mutation of some genes, such as KCNQ1, KCNE2, KCNJ2 and KCNH2, is connected to the increase in the function of potassium channels, which interferes with the refractory period of the potential of action of cardiomyocytes, predisposing to arrhythmia. Recently, other studies identified the association of more genes with AF (SCN5A, KCNA5, NPPA, NUP155 and GJA5), given that the mechanism for the onset of arrhythmia is still being elucidated.

In the clinical practice, the fact that some individuals enduring a myocardial ischemic event present more propensity to the condition of ventricular fibrillation and sudden cardiac death is well established. Some studies have been pointing to genetic predisposition to this phenomenon, such as the prospective study PARIS and have been demonstrating that chromosomal variants located in chromosome 21q21, and possibly in chromosome 9q21, may make these patients susceptible due to changes to ion channels. Monogenic diseases of ion channels (channelopathies) have been much studied over the last decades. The Long QT Syndrome has been demonstrated to be associated with mutations of SCN5A, KCNH2 and, more recently, KCNQ1, and KCNE1; and pharmacogenetic approach has been proposed for the treatment directed at each type of gene involved.

The Brugada Syndrome, described in November 1992, when identifying an electrocardiographic pattern of right bundle branch block in eight individuals with clinical and family history of sudden death and a structurally normal heart, characterizing an electroclinical phenotype, was one of the examples of how the molecular biology over the last decades allowed understanding channelopathies and treat them based on genetic patterns. Therefore, the use of the systematic approach employing systems biology in cardiac arrhythmias is in its early stage and may in the future help develop new treatments focused on the pathophysiological target of the abnormality present in each specific type of arrhythmia.

Heart failure

The cardiomyopathies represent an heterogeneous and complex group of diseases bringing on impairment of myocardium and having a family / genetic, inflammatory, infective, toxic or idiopathic cause. From the anatomico-physiological standpoint, cardiomyopathies may have three classical phenotypes: the dilated form, the hypertrophic model and the restrictive pattern. Currently, the restrictive form allows the search for an etiology with the employment of methods of heart imaging, genetic tests and endomyocardial biopsy. The heart impairment by amyloidosis, although a rare form of HF, has been more recently addressed with views based on the omic sciences, allowing identifying mutations in different proteins responsible for the formation of the amyloid protein, correlating it with the clinical presentation, establishing prognosis and guiding the specific treatment.
In patients with suspected hereditary amyloidosis, the molecular imaging through SPECT myocardial scintigraphy with DPD-Technetium-99m identifies the amyloid deposition and, with the aid of molecular biology and proteomics techniques, it is possible to define the type of material existing in the fibril of amyloid involving the transtirretin (TTR). The treatment with the drug Tafamidis, by stabilizing the tetrameric form reducing the formation of amyloid fibril, seems to delay the progression of the amyloid cardiomyopathy\textsuperscript{43,44}. Besides this, new drugs which work silencing the RNA and therapies with antisense oligonucleotides, which reduce the production of TTR, are being studied in several clinical trials\textsuperscript{45} (Figure 6).

Limitations

The high cost of the omic sciences and the difficult access, as well as the theoretical knowledge of systems biology are limiting aspects in Brazil for the development of cardiovascular researches and the approach to patients under this new paradigm.

Future prospects

The systems biology has the potential to further the understanding of the interaction of different metabolic routes involved in the etiopathophysiology and phenotypes of cardiovascular diseases. Therefore, this new approach may be useful to reclassify the cardiovascular diseases and develop new therapeutical targets, as has been demonstrated with the microRNAs and, progressively, cement the personalized cardiovascular medicine.

Acknowledgement

We would like to thank Prof. Cláudio Tinoco Mesquita and Oswaldo Nascimento for the molecular imaging in this manuscript.

Potential Conflicts of Interest

No relevant conflicts of interest.

Sources of Funding

This study had no external funding sources.

Academic Association

This manuscript is part of the activities of the Cardiovascular Sciences Graduate Program at UFF.

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