New cardiovascular biomarkers in animal models: what can be expected in the coming years?

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Animal models of disease provide the basis for important scientific inquiry of proof of concept, drug safety and efficacy, and exploration of the mechanistic and pathophysiological aspects of disease. Animals with either induced cardiac disease or spontaneously occurring cardiac disease are test beds for new cardiovascular therapies or interventions. Biomarkers in animal models can help characterize disease, stratify risk of morbidity and mortality, and serve as surrogate endpoints. Biomarkers identify important similarities and differences between the animal model and the human condition. The future of biomarkers and animal models includes cross-organ investigation using multiorgan multimarker panels. Industry will use animal models and biomarkers to help shorten the time from drug discovery to market. Biomarker development has outpaced their acceptance in clinical use and carefully designed clinical studies are needed to achieve their maximum potential. ***Cardiovasc Endocrinol*** **3:27–31** © **2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Animal models of disease provide the basis for important scientific inquiry of proof of concept, drug safety and efficacy, and exploration of the mechanistic and pathophysiological aspects of disease. In particular, studies using animals with either induced cardiac disease or spontaneously occurring cardiac disease (SOCD) are of interest insofar as cardiovascular disease represents one of the leading causes of death and disability worldwide. The potential advantages of induced disease models include their neurohormonal and functional characteristics similar to human heart failure, reliability, and presence of a large amount of previously published data using these models. Commonly used induced models of myocardial or valvular disease include rapid ventricular pacing [1], microembolization [2], and chordae tendineae rupture [3]. The potential advantages of animals with SOCD include the ability to test interventions in subjects with the actual disease of interest, access to a large number of affected subjects, and avoidance of ethical and animal welfare considerations that accompany induced disease models. SOCD is relatively common in veterinary species such as the dog, and particular forms of SOCD in dogs are highly homologous to the corresponding human disease. Examples include myocardial diseases such as dilated cardiomyopathy [4,5] and arrhythmogenic right ventricular cardiomyopathy (ARVC) [6] and valvular diseases such as mitral valve prolapse [7] and myxomatous mitral valve disease (MMVD) [8]. Irrespective of whichever model is utilized, characterization of the disease state in individual subjects must be comprehensive, valid, and reliable. This is typically easier to accomplish in induced disease models where the subjects are often of the same age, sex and breed, and whose diet, environment, and activity level can be closely controlled. The obvious disadvantage of induced disease models, from a biological standpoint, is that while similar, the induced disease often has important differences with respect to the rate of onset and degree of ventricular remodeling to the actual disease of interest.

Biomarkers are objective measures that predict the presence, risk, or outcome of disease. As such, they act as surrogates for clinically relevant conditions (i.e. presence of disease) or outcomes (i.e. heart failure, death). Cardiovascular biomarkers are increasingly being used to help diagnose, stage, monitor, and predict outcome of cardiovascular disease in both humans and animals [9,10]. The advantages of using biomarkers include their objectivity, potential cost-effectiveness, and in many cases, their pathophysiological link with the disease of interest, such that their study yields a deeper understanding of the mechanisms of disease [11]. Industry uses biomarkers as surrogate markers in animal models to help speed the development of new drugs, especially in diseases that progress slowly. The number and nature of potential cardiovascular biomarkers under investigation are both plentiful and diverse. This review will discuss future veterinary trends involving cardiovascular biomarkers and briefly describe some of the promising biomarkers under study in both human and animal models.

Cardiac biomarkers in animal models of cardiovascular disease: 10 years hence

What will the use of biomarkers in animal models of disease look like 10 years into the future? What are the

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most promising applications and where should we expend the majority of our resources to maximize return? As stated previously, induced disease animal models, by applying a uniform cardiac injury to each subject, offer homogeneity with respect to the severity and expected progression of disease across individuals. By controlling these variables, extraneous noise is reduced and the ability to make inferences on the effects of a treatment, for example, is increased. In contrast, SOCD models by nature are much more heterogeneous as the affected population includes individuals of varying ages, sexes, body habitus, severity of disease, duration of disease, and risk of future adverse events. These potential confounders can obscure the effect of an intervention and increase the number of subjects that must be studied.

If eligible subjects with SOCD could be better stratified into groups with similar clinical and biochemical characteristics, or if individuals at highest risk for adverse events could be selected from among the entire population, the use of SOCD models would be highly attractive. To illustrate this point, consider the case of a SOCD such as MMVD. In dogs, the clinical spectrum of MMVD ranges from mild mitral valve prolapse to ruptured chordae tendineae and torrential mitral regurgitation. In animals with preclinical MMVD, the rate of disease progression is highly variable and difficult to predict. In veterinary species with SOCD, biomarkers provide insights into the severity of disease, risk of morbidity and mortality, and response to therapy, such that populations of diseased individuals can be subdivided into groups with similar disease characteristics, risks, and outcomes [12,13]. Heart disease represents a significant societal burden with respect to healthcare costs and resources, and identification of individuals at highest risk for disease progression is of interest. The prediction of adverse events such as hospitalization for heart failure in an individual patient, be it dog or human, is difficult. The cardiac natriuretic peptide NT-proBNP, as has been discussed previously in this issue, stratifies the risk of morbidity and mortality in both dogs and humans. Characterization of study populations partly based on NT-proBNP or other relevant biomarkers could facilitate the study of a specific predisposed subgroup of humans by leveraging the ability to closely match them to their high-risk canine counterparts.

Biomarkers can also highlight the differences between SOCD and the corresponding human disease. An example is illustrated by ARVC. ARVC in the dog shows a high degree of clinical homology with human ARVC [6]. Both conditions are characterized by a high incidence of ventricular tachycardia, syncope, and sudden death, and variable degrees of myocardial failure and congestive heart failure. ARVC is particularly common in the Boxer breed of dog and is diagnosed on the basis of clinical signs of syncope and evidence of ventricular arrhythmias with right bundle branch block morphology [6]. The close clinical features between the two species suggest that new antiarrhythmic strategies in the dog could lead to better outcomes in humans. In the Boxer dog, a single genetic mutation associated with ARVC has been described [14]. However, this particular mutation, involving the *striatin* gene, is not one of the 12 different genetic subtypes of ARVC that have been described in humans. Here, the difference in biomarkers can be considered a glass half-empty (are findings from Boxer ARVC studies really applicable to humans?) or half-full (the potential for mutations in the *striatin* gene should be evaluated in humans with ARVC).

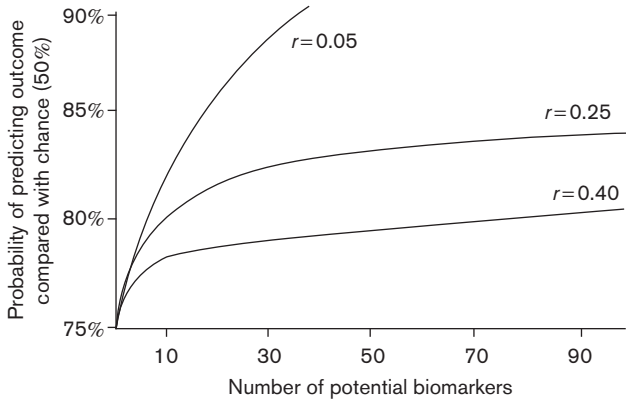
One trend in biomarker science likely to encompass both animal and human disease is the use of multiple complementary markers for a particular disease entity. The value of simultaneously assessing multiple biomarkers can be additive or synergistic. A study has shown that NT-proBNP and cardiac troponin-I provide complementary prognostic information in dogs with SOCD [12]. Dogs with elevations of both NT-proBNP and cardiac troponin-I fare worse than those with elevations of either biomarker alone. This latter group fares worse than dogs with neither biomarker elevated. In human patients, the multimarker approach has proven valuable in heart failure [15,16], myocardial infarction [17], and acute dyspnea [18]. Thus, carefully designed 'biomarker panels' that provide a cross-sectional profile of multiple markers will likely provide superior information than testing a single marker alone. Some of the most promising markers being studied in animal models and humans are listed in Table 1. These markers are grouped under broad headings that reflect their purported role in the pathophysiology of disease. Panels that include a sampling of markers across different pathophysiological categories likely provide superior information than testing multiple markers with a high degree of correlation within categories. The multimarker strategy for predicting likelihood of a theoretical outcome is most discriminative if their individual components, while all reflecting important aspects of the disease process, are only weakly correlative to each other (Fig. 1). This strategy is similar to looking at the disease from a variety of different 'viewpoints' in the hope of getting the most complete and accurate picture.

Regulation of the cardiovascular system involves a complex interplay between many different organ systems, including the heart, kidney, vasculature, and autonomic nervous system, and biomarker panels involving more than a single organ system are a subject of considerable interest. In diseases such as congestive heart failure or acute kidney injury, biomarkers with a wider 'cardiorenal' perspective have been proposed. Cardiorenal syndrome has been described as 'disorders of the heart and kidneys whereby acute or chronic dysfunction in one organ may

Table 1 Incomplete list of potential circulating biomarkers in animal models and humans with cardiovascular disease

Acute and chronic heart failure
NT-proBNP/BNP
cTnI/hscTnI
Galectin-3
ST-2
Mid-regional proadrenomedullin
Copeptin
sST-2
Mid-regional proatrial natriuretic peptide (MR-proANP)
MicroRNAs (mRNAs)
Growth differentiation factor-15 (GDF-15)
Extracellular matrix
Soluble endoglin
Osteopontin
PIIINP
MMPs
Glycoprotein 130 (gp130)
Inflammatory
Heat shock protein
Vascular cell adhesion molecule-1 (VCAM-1)
C-reactive protein
Interleukin-1
Metabolism
Leptin
Branched chain amino acid metabolites
Cardiorenal disease
Neutrophil gelatinase-associated lipocalin (NGAL)
Cystatin C
Kidney injury molecule-1 (KIM-1)

Fig. 1



Incremental value in adding potential biomarkers of varying marker–marker correlation (r). The y-axis shows the probability of the hypothetical outcome measure as compared with chance (50% probability). The x-axis shows the number of markers used to help determine the probability of the outcome. The greatest incremental gain in the probability occurs when the markers used are only weakly correlated with each other ($r=0.05$), as opposed to a lesser amount of gain if the markers are more strongly correlated to each other ($r=0.25$ or $r=0.40$). Adapted from Roberts *et al.* [19].

induce acute or chronic dysfunction of the other' [20]. Several cardiorenal biomarkers (Table 1) have been proposed as a means to specifically identify cardiorenal syndrome, stratify injury severity, and to guide subsequent therapy. New and existing markers will undoubtedly undergo testing in animal models of disease. In the author's opinion, a multiorgan multimarker strategy is one of the most tantalizing aspects of biomarker studies.

The science of new biomarkers in both animals and humans is evolving rapidly. Contributions to this evolution involve a variety of interested parties. Biopharmaceutical companies are interested in using biomarkers to assess toxicity as well as surrogate endpoints to speed drug development for slowly progressing diseases [21]. Physicians and veterinarians seek biomarkers that identify high-risk patients or patients who are most likely to respond favorably to a given therapy. Diagnostic testing companies are eager to bring new discoveries into the market. These and other forces are driving the search for new cardiovascular biomarkers, and the pace of discovery has largely overtaken the rate of acceptance. The excitement surrounding newly discovered biomarkers needs to be justified through carefully designed studies that validate the benefit of these markers in clinical practice.

New cardiovascular biomarkers

A broad variety of molecules involved in heart failure, tissue remodeling, inflammation, the extracellular matrix, and metabolism are being studied. An exhaustive review of emerging biomarkers is beyond the scope of this article, but a selection of some intriguing molecules is now presented.

MicroRNA

MicroRNA (miRNA) are short noncoding RNAs that block translation and speed breakdown of specific target messenger RNA. miRNA play important roles in many different disease processes including cardiovascular, kidney, neoplastic, and neurologic disease. miRNA help regulate a variety of aspects of cardiovascular function including hypertrophy, fibrosis, apoptosis, cardiac conduction, and energy utilization [22]. An example of miRNA in cardiac disease involves atrial fibrillation. A well-established animal model of atrial fibrillation is induced atrial fibrillation in dogs [23]. In affected dogs, miRNA-29 and miRNA-26 are downregulated and contribute toward pathological fibrotic atrial remodeling [24] and overexpression of the inward-rectifying K+ current [25], features that are important characteristics of atrial fibrillation in humans. Recently, the study of miRNA in heart disease has included dogs with SOCD [26,27].

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) and their inhibitors belong to a family of endopeptidases capable of degrading and remodeling extracellular matrix. MMPs are involved in a wide variety of cell functions including apoptosis, differentiation, and proliferation. A role for MMPs in cardiac disease is well established in humans [28], induced-disease models [29,30], and SOCD [31,32]. Animal models of disease will be useful in determining whether pharmacological modulation of MMP activity can reduce pathological cardiac remodeling [33].

Leptin

Leptin is an adipose tissue-derived hormone that helps regulate energy expenditure, metabolism, and hunger. Leptin's effect on the cardiovascular system can be direct, through its effects on cardiomyocyte fatty acid utilization, hypertrophy, inflammation, and fibrosis, as well as indirect, through effects on renal function, insulin resistance, and body weight [34]. In dogs with SOCD, leptin gene transcription was increased in the myocardium and blood compared with healthy controls, and was highest in the dogs with the most severe heart failure [35]. In human patients, leptin expression has been associated with increased morbidity and mortality [36]. This effect is mediated, in part, by activation of inflammatory cytokines and increased redox stress within myocardiocytes [37].

Neutrophil gelatinase-associated lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is a glycoprotein that is released by renal tubular cells secondary to kidney injury. Once in circulation, NGAL promotes apoptosis and inflammation of other organs, including the heart. In one study of human patients, NGAL was superior to NT-proBNP in predicting adverse cardiac events of acute decompensated heart failure within 30 days of discharge from the hospital [38]. Moreover, patients with elevations of both NGAL and NT-proBNP were identified to be at highest risk, indicating the potential advantage of a multimarker strategy. In another study, elevated urinary NGAL in patients being treated for acute heart failure was a superior determinant of worsening renal function at 72–96 h versus conventional means [39]. Elevated urinary NGAL has been reported in dogs with both spontaneous and acute kidney injury [40,41].

In summary, the use of biomarkers in animal models of disease is likely to increase. Testing of biomarkers in animals with SOCD will likely grow as awareness of these models increases within the medical community. Multimarker multiorgan approaches are particularly intriguing. The best chance of continuing success within the field of biomarkers will require cooperation between many different parties, including veterinary and medical researchers and clinicians and industry.

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Conflicts of interest

There are no conflicts of interest.

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