1st Human and Veterinary Crossover Symposium on Aldosterone

Bordeaux
16th-17th-18th October 2009
CEVA Santé Animale is very pleased to welcome you to the 1st Human and Veterinary Crossover Symposium on Aldosterone

"Place de la Bourse. City of Bordeaux. France."

PREFACE

The human – animal bond is an essential part of all our lives, which equally needs to be embraced in science.

We are delighted to have hosted what we titled the “First human and veterinary crossover symposium on Aldosterone”, in our home area of Bordeaux, France.

The development of canine cardiology is a new and exciting area and one which clearly illustrates the advantages of exchanging expertise between the human and veterinary fields.

During the production of our 2008 annual report, we set out to better understand what if anything Ceva could contribute to the major animal health challenges that we face globally.

The spread of zoonotic diseases, with the recent outbreaks of the so called “swine flu” and the currently more localised emergence of Q-fever in Holland have brought “animal health” to the forefront of the media. The crossover between human and animal medicine has of course a very long history, from the work of Edward Jenner in 1796 on small pox through to Pasteur almost 100 years later, there are many examples of scientists who were able to cross the gap.

Innovation is one of Ceva’s values, many other companies have also adopted this word as a description of what they do and so it is very important that as a group of individuals we clearly understand what we mean by the word.

Innovation is not simply about finding “blockbuster” new molecules; we have to encourage thinking outside the conventional project-style approach, move the lines, promote cross-fertilisation of ideas. The English proverb “necessity is the mother of invention”, probably typifies our approach at Ceva, we don’t necessarily have the largest resources to commit to R&D (although we have consistently increased our spending in this area, throughout our 10 year history) and so we must ensure that what resource we commit will generate results. I believe that our work, in collaboration with the foremost global experts, in looking at aldosterone as a blockade in the treatment of chronic heart conditions, is a good example of selective reasoning, applied to product development.

Another one of Ceva’s values is that of entrepreneurial spirit. Science is not often associated with entrepreneurship but a report published earlier in the year in the Economist magazine described it as – “somebody who offers an innovative solution to a (frequently unrecognised problem)”. In this sense, it is important that our own scientists together with their network of scientific partners, move quickly and take the reasoned risks and decisions in order to improve our knowledge of animal health.

We are delighted that so many of you as the leading experts in the field of cardiology accepted our invitation to participate and share their knowledge in this event. I give you my personal assurance that Ceva will commit as much resource – be it human or financial, as we can to ensure that we make further advancement in the development of canine cardiology.

The way that we interact with animals is central to our lives, as scientists we have a responsibility to help better protect them and as humans to enjoy their company and have fun with them.

Marc Prikazsky
President and CEO, Ceva Santé Animale
Mark Oyama is an Associate Professor in the Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania. His main clinical and research interests involve canine myocardial disease, mitral valve disease and cardiac biomarkers. He is currently involved in several research projects dealing with investigation of biomarkers in dogs and cats as well as studies investigating serotonin-related molecular mechanisms of canine valve disease.

He is the current President of the American College of Veterinary Internal Medicine, Specialty of Cardiology and a member of the ACVIM Board of Regents. He has served as a member of the NIH-Center for Scientific Review Bioengineering, Technology, and Surgical Sciences Study Section and is a member of the University of Pennsylvania Institute of Translational Medicine and Therapeutics. Dr. Oyama has published over 90 scientific manuscripts and abstracts and has given over 150 national and international lectures. He serves on veterinary advisory boards for a variety of biotechnology, pharmaceutical, and diagnostic laboratory companies and is the Translational Sciences section editor for the Journal of Veterinary Cardiology. He resides in Philadelphia with a Golden retriever and two very mischievous cats.

Jonathan Elliott graduated from Cambridge Veterinary School in 1985. After a year as an Intern at the University of Pennsylvania, he undertook a PhD in vascular pharmacology in Cambridge.

In 1990 he was appointed to a lecturership in Veterinary Pharmacology at the Royal Veterinary College and developed research interests in feline kidney disease and hypertension and Equine Laminitis. He was awarded the Pfizer Academic Award in 1998 and the BSAVA Amoroso Award in 2001, the Petplan Scientific Award in 2005 and the ESVNU Award in 2007 for contributions to companion animal medicine. He was a member of the ACVIM Consensus Statement Panels on Proteinuria and Hypertension and chaired the International Renal Interest Society from 2002-2004.

He is currently Professor in Veterinary Clinical Pharmacology and Vice Principal for Research at the RVC and is a Diplomate of the European College of Pharmacology and Toxicology and a member of the UK Government’s Veterinary Products Committee.

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10:30 - 11:00 Coffee-break
11:00 - 12:30
Mineralocorticoid receptor blockade: how experimental evidence supports the clinical benefit.
Johann Bauersachs

The proximate cause of heart failure: models, remodeling, and re-remodeling.
Robert L. Hamlin

12:30 - 14:00 Lunch

14:00 - 16:30
After RALES, the journey continues.
Bertram Pitt

Efficacy of spironolactone in dogs with naturally occurring Myxomatous Mitral Valve Disease.
Claudio Bussadori

Principles of survival analysis illustrated by CEVA’s spironolactone trials.
John Martin Bland

16:30 - 16:50 Coffee-break
16:50 - 17:30
Current clinical status and future directions for biomarkers in heart failure.
Faiez Zannad

17:30 - 17:45
Closing the symposium
Nicolette Farman is Research Director (Senior Investigator) at INSERM (French National Institute of Health and Medical Research). She joined the public research body in 1978 (INSERM U2, Limeil Brévannes Hospital) and was a Board Member between 2001 and 2008.

She is a Member of the French Society of Nephrology, American Society of Nephrology, American Physiological Society and Honorary Member of the South-American Society of Nephrology. She is referee for several peer-review journals such as J. Am Soc. Nephrology, Am. J. Kidney Diseases, Endocrinology, Nature.

Nicolette Farman has organized four international congresses dealing with aldosterone in France and abroad. She was one of the pioneers in discovering the cardio-vascular effects of this hormone.

ALDOSTERONE AND MINERALOCORTICOID RECEPTORS: EXPANDING VIEWS FROM THE KIDNEY TO THE CARDIOVASCULAR SYSTEM

The steroid hormone aldosterone plays a major role in the control of blood pressure and extracellular volume homeostasis. Aldosterone is synthesized in the glomerular zone of the adrenal cortex in response to hyperkalemia or sodium depletion, as the endpoint of activation of the renin-angiotensin system. Considerable work has been achieved to improve the understanding of the molecular and cellular events involved in renal aldosterone actions, in normal as well as in pathological situations.

It is well established that aldosterone stimulates renal sodium reabsorption and potassium excretion, an effect that can be blocked by administration of the mineralocorticoid receptor (MR) antagonist spironolactone. These effects are observed 1-2hrs after hormone administration, a delay required for hormonally-mediated activation of sodium transporters and regulation of transcription of target genes. Aldosterone binds to the MR, which is a ligand-dependent transcription factor that belongs to the nuclear receptor superfamily. Once translocated into the nucleus, hormone-receptor complexes bind to glucocorticoid response elements within the promoter region of early aldosterone-induced genes to modulate their transcription. This triggers an increase in the activity and number of sodium transporters or channels. In a typical target cell for aldosterone of the distal nephron, such as the renal collecting duct principal cell, the sodium of the tubular fluid enters the cell through amiloride-sensitive apical sodium channels, and it then extruded from the cell to the peritubular space by the Na-KATPase located in the basolateral membrane.

MR antagonists (spironolactone, eplerenone) prevent aldosterone binding and thus are useful therapeutic tools to prevent inappropriate MR activation. The MR has been cloned in 1987, and it was evidenced that the MR displays similar high affinity (in the nanomolar range) for aldosterone and glucocorticoid hormones, that are much more abundant in the plasma (100-1000 fold) than aldosterone. This should lead to permanent illicit occupancy of the MR by glucocorticoid hormones, inducing permanent maximal sodium retention, independent of plasma aldosterone levels. The MR protector enzyme 11 ß hydroxysteroid dehydrogenase type 2 (11-HSD2, coexpressed with the MR in cells of the distal nephron) metabolizes circulating glucocorticoid hormones (cortisol in man, corticosterone in rodents) into inactive 11-dehydro derivatives (cortisone, 11-dehydrocorticosterone) with very low affinity for the MR.

More recently it became apparent that the MR is also expressed in the cardio-vascular system, together with low HSD2 and the nature of aldosterone action in these tissues is actively investigated, in normal as well as in pathological situations.
Beside the cardiovascular consequences of an altered functioning of the renin-angiotensin system leading to hypertension, direct cardiovascular effects of aldosterone have been evidenced. There are many data in favour of an involvement of aldosterone in cardiac remodelling and fibrosis. Exposure of uninephrectomized rats to aldosterone and high salt diet leads to cardiac fibrosis that can be prevented by spironolactone. Importantly, the RALES and the EPHESUS studies have demonstrated the beneficial effect of MR-antagonists (spironolactone, eplerenone) in large cohorts of patients with severe heart failure or following myocardial infarction. These initial observations have now opened a major new field to improve treatments of cardiac failure in patients. Major efforts are now made to elucidate aldosterone impacts on cardiovascular functions.

References

OVEREXPRESSION OF THE MINERALOCORTICOID RECEPTOR: PATHOPHYSIOLOGICAL CONSEQUENCES

1- Mineralocorticoids and Cardiovascular diseases

Aldosterone (Aldol) is the main regulator of sodium reabsorption. The mineralocorticoid receptor (MR) is activated by Aldo binding and is targeted to the nucleus where it binds to Glucocorticoid (Gluco) Responsive Elements and triggers gene transcription. New targets of Aldo have been discovered in the last decade, that extends its actions to the cardiovascular (CV) system. We have shown that MR is expressed in cardiomyocytes, coronary vessels, aorta and resistance arteries. MR is expressed in both endothelial and smooth muscle cells where its functions are still poorly understood. Vascular MR activation, as addressed in pharmacological experimental models or in human volunteers and patients with CV diseases, has been proposed to be linked to endothelial dysfunction and vascular wall remodeling.

The RALES and EPHESUS clinical trials have demonstrated that the addition of MR antagonists to standard care markedly reduce the overall and cardiovascular mortality in patients with heart failure or in acute myocardial infarction complicated by left ventricular dysfunction, respectively. Moreover, MR antagonism has been recently shown to be beneficial in diabetic nephropathy, as well as in chronic renal diseases. The beneficial effects of MR antagonists in CV diseases are explained mainly by a decrease of cardiac fibrosis and an improvement of peripheral vascular function. However, direct effect on cardiac myocytes, endothelium and smooth muscle cells are still underscored.

Plasma Aldo levels are not correlated with the clinical response to MR antagonists. This may be explained by an increase in MR expression/MR activation in heart, vessel and kidney as reported in human diseases and experimental animal models. MR expression is increased in heart after myocardial infarction, heart failure and hypertension. In the vessels MR expression is increased in arterioles of SHR rats.

MR activation (in absence of increased Aldo or MR expression) has also been proposed to be related to cellular oxidative stress.

The signaling pathways involved in mineralocorticoid effects in CV diseases remain elusive because: 1) most of the proposed signaling pathways have been identified in cultured cells; 2) their identification using in vivo pharmacological approaches (aldosterone infusion, MR antagonism) is hampered by the pleiotropic effects of Aldo and MR activation, mixing primary events with the confounding secondary responses; 3) in vitro, the MR can bind with similar affinities both Aldo and Gluco. Thus, analyzing the specificity of Aldo/Gluco/ MR signaling pathways is fundamental to understand their pathophysiological roles in the CV system.

Very few genes have been reported to be modulated by MR activation in the endothelium, mainly based on...
short-term Aldo treatment of cultured cells. 

Activation of MAP kinases pathways has been reported in vascular smooth muscle cells, as well as induction of genes involved in vascular fibrosis, inflammation and calcifications (see for review 14). So far, biomarkers of MR activation have not been identified.

2- Conditional transgenic models: a clue to understand specific molecular and functional roles of MR activation in the heart and vessels

The profibrotic effect of aldosterone has been first identified in 1990 by Brilla and Weber in a pharmacological model combining uninephrectomy, high salt diet and infusion of aldosterone (or DOCA, a mineralocorticoid analogue) 15. Both right and left ventricles presented with interstitial remodelling suggesting that this was related to humoral disorders but not hemodynamic alterations due to high blood pressure. Indeed administration of a low dose of spironolactone, which was unable to prevent blood pressure increase, prevented cardiac and vascular remodelling as well as local inflammation 16-18. This confirmed that the deleterious effects were not related to hemodynamic factors but to local Aldo/MR activation.

Experimental models, such as non-targeted MR gene inactivation or pharmacological models (manipulating ligands or MR/GR antagonists) are complex to analyze. The pleiotropic effects of Aldo and Gluco as well as the ligands or MR/GR antagonists) are complex to analyze. The targeted approach allows spatio-temporal control of MR and GR expression, making difficult to unravel their specific contributions to large tissue distribution of their respective receptors.

Pathophysiological role of Aldo and Gluco as well as the targeted approach (with interstitial remodelling) suggest that this was related to humoral disorders but not hemodynamic alterations due to high blood pressure. Indeed administration of a low dose of spironolactone, which was unable to prevent blood pressure increase, prevented cardiac and vascular remodelling as well as local inflammation. This confirmed that the deleterious effects were not related to hemodynamic factors but to local Aldo/MR activation.

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Several double-transgenic mouse models with conditional, inducible manipulation of MR and GR expression have been generated and functionally analyzed in our laboratory. Such animal models provide tools that allowed to progress in the understanding of the steroid-mediated pathophysiology. This allows to address: i) Molecular and functional effects of MR in each target organ/cell type ii) In integrated in vivo responses permitting the analysis of both primary and secondary effects iii) Combination of genetic and pharmacological approaches to define specificity of the responses and of the identified pathways.

Pathophysiological role of MR/GR in the heart

1- Cardiac MR over-expression led to major electrocardiographic abnormalities with prolonged ventricular repolarisation and spontaneous and triggered ventricular arrhythmias. This was associated with ion channel remodelling (patch-clamp) leading to a decrease in Ito K current and an increase in action potential duration and Ca transient amplitude. During embryogenesis and adulthood, mice exhibited a high rate of death prevented by the MR antagonist spironolactone 20.

2- A cardiac GR over-expressing model shows several phenotypic differences as compared to the MR model, with major conduction defects and a mild dilated cardiomyopathy, but no embryonic lethality. Ion channel remodelling also differed, indicating that specific pathways are distinctly regulated via MR or GR activation in the heart 21.

Pathophysiological role of MR in the vessels

In order to manipulate Aldo/Gluco and MR/GR signalling in the vessels, MR over-expression has been recently achieved in endothelial cells using an endothelial-specific transactivator strain. Increased MR activation in endothelial cells only is associated with increased blood pressure (independently of alterations in ion homeostasis), altered vascular reactivity as estimated ex vivo in mesenteric arteries and altered in vivo blood pressure response to vasoconstrictors like AngII and ET1.

Identification of specific gene targets of the MR

Differential screening of gene targets specifically modulated upon MR activation but not GR activation was done in the heart of the MR/GR conditional models. Using transcriptome analysis done in heart samples from mice with conditional overexpression of either MR or GR in the cardiomyocytes only, we analyzed differential expression of 10 000 mouse genes (using MWG microarrays) as well as 6000 genes selected for their putative implication in heart diseases (these genes were selected based on their expression in cardiac tissue affected by various pathologies). Some of the genes overlapped between the two arrays. We were able to identify networks of genes specifically up- or down-regulated in the MR or GR model. Several candidates were selected from these results and validated in individual mice at different time points after MR/GR expression. About 15 genes that were up- or down- regulated in the MR model (as compared to control mice and GR mice) were validated.

Some of them have been proposed as putative biomarkers of MR activation since they encode secreted protein that have been identified in the plasma of these transgenic models as well as in a rat heart failure model. Interestingly, pharmacological MR antagonism normalized the increased plasma level of these biomarkers.

Taken together, our data indicate that:

-Our unique models are powerful tools to identify and validate biomarkers of MR activation.
-Such biomarkers of MR activation may be particularly useful in human and canine patients with heart failure, human patients with infarct, metabolic syndrome (including diabetes), as companion diagnostic in order to adapt the treatment on an individual basis. Indeed we have shown that one of these markers is also expressed in the dog but its relevance in canine heart failure remains to be studied.
References


Johann Bauersachs is an Associate Professor at the University Hospital of Würzburg (Germany) in the Division of Cardiology. He has Board Certifications in Internal Medicine Cardiology and Critical Care. He received two Awards from the German Society of Cardiology (2001, 2004) and more recently the Parmley-Award of the American College of Cardiology in 2007.

His current research topics are healing and remodeling after myocardial infarction; cardiac ischemia- and reperfusion injury; cardiac regeneration by progenitor cells; endothelial progenitor cells, endothelial dysfunction and platelet activation in coronary artery disease, myocardial infarction, hypertension and diabetes mellitus.

He is member of the Editorial Board of Cardiovascular Research, European Journal of Clinical Investigation, and Basic Research in Cardiology.

Johann Bauersachs is the President of the European Section of the Aldosterone Council (ESAC) in Germany since 2006.

MINERALOCORTICOID RECEPTOR BLOCKADE: HOW EXPERIMENTAL EVIDENCE SUPPORTS THE CLINICAL BENEFIT

Activation of the renin-angiotensin-aldosterone system plays an important role in the pathogenesis of cardiovascular disease. High levels of aldosterone impair cardiac and vascular function and predict mortality risk of patients with acute myocardial infarction or heart failure. Patients with primary hyperaldosteronism display a higher incidence of myocardial infarction and stroke. Large clinical trials (RALES, EPHESUS) have shown mineralocorticoid receptor (MR) blockade to decrease mortality in heart failure

In contrast to the classical notion that mineralocorticoids were only involved in body electrolyte and water homeostasis mediated by the kidney, aldosterone exerts important direct (patho)physiological effects on the cardiovascular system. While the extra-adrenal generation of aldosterone from heart and vessels appears to be of minor importance, MR expression has been repeatedly documented in cardiovascular cells. Aldosterone targets MR on cardiomyocytes, cardiac fibroblasts, endothelial cells and smooth muscle cells leading to cardiac hypertrophy, fibrosis and vascular injury. MR expression in the heart is increased in heart failure explaining detrimental effects of aldosterone even in the absence of markedly elevated circulating levels of aldosterone. Under certain pathophysiological circumstances, also glucocorticoids may act as agonists on the MR. Upon binding to the MR aldosterone increases cardiomyocyte as well as vascular endothelial and smooth muscle cell reactive oxygen species formation.

In dogs with chronic heart failure induced by microembolization, long-term treatment with the MR blocker eplerenone attenuated progressive left ventricular (LV) dysfunction as well as LV dilatation, hypertrophy and fibrosis. In dogs with heart failure induced by rapid pacing, spironolactone reduced atrial structural and electric remodelling, associated with significantly less and shorter episodes of atrial fibrillation.

Long-term MR blockade with eplerenone and angiotensin converting enzyme (ACE) inhibition alone or in combination were studied in rats with heart failure after extensive myocardial infarction. Like ACE inhibition, eplerenone attenuated LV filling pressure and volume. Combination therapy significantly improved LV systolic and diastolic function, and reduced LV end-diastolic volume. In parallel, molecular alterations such as changes in collagen expression or myosin heavy chain isoforms were reversed by combination therapy.

In organ baths studies, the effect of MR blockade on endothelial function was assessed using phenylephrine-precontracted aortic rings from rats with chronic heart failure. Long-term treatment with spironolactone in addition to
ACE inhibition normalized the acetylcholine-induced nitric oxide-mediated relaxation which was significantly attenuated in rats treated with placebo, and prevented the increase in superoxide anion formation. MR blockade also attenuated the platelet activation observed in rats with heart failure, an effect which may also translate into reduction of thrombo-embolic events in patients with heart failure. 10,11

To clarify that indeed direct effects in the heart mediate beneficial actions of MR blockade, we investigated LV remodelling after myocardial infarction in mice with cardiomyocyte-specific deletion of the MR gene (MR<sup>−/−</sup>) which were generated using a conditional MR allele MR<sup>flx</sup> in combination with a transgene expressing Cre recombinase under control of the myosin light chain (MLD2a) gene promoter. Sham-operated wildtype and MR<sup>−/−</sup> mice showed similar cardiac morphology and function. LV function was preserved and LV dilatation was attenuated in the MR<sup>−/−</sup> mice with chronic heart failure compared to wildtype animals. This was associated with reduced superoxide formation, myocyte cross-sectional area and collagen accumulation in the surviving myocardium. Thus, myocyte-specific deletion of the MR gene protects against adverse cardiac remodeling and contractile dysfunction in ischemic heart failure, suggesting that clinical benefits of MR blockade in patients with heart failure may be mediated at least in part via a cardiomyocyte-dependent mechanism.

As bone marrow derived endothelial progenitor cells (EPC) play an important role in endothelial repair and angiogenesis, we hypothesized that hyperaldosteronism impairs EPC function and vascularisation capacity in mice and humans. Treatment of human EPC with aldosterone induced translocation of the MR and impaired multiple cellular functions of EPC, such as differentiation, migration, and proliferation in vitro. Aldosterone protein kinase A-dependently increased reactive oxygen species formation in EPC. Aldosterone infusion in mice impaired EPC function and vascularisation capacity in a MR-dependent manner. EPC from patients with primary hyperaldosteronism compared to age-matched controls displayed reduced migratory potential. MR blockade in patients with hyperaldosteronism improved EPC function as well as endothelial function. Thus, normalization of EPC function may represent a novel mechanism contributing to the beneficial effects of MR blockade in heart failure and other cardiovascular diseases.

In the Würzburg heart failure registry including consecutive heart failure patients with either preserved or reduced LV function, higher serum levels of both cortisol and aldosterone were independent predictors of increased mortality risk conferring complementary and incremental prognostic value. While numerous studies suggest that MR blockade is also effective in heart failure with preserved ejection fraction, definitive evidence will come from ongoing randomised, placebo-controlled studies. 12

In conclusion, there is ample evidence from experimental and clinical trials that mineralocorticoid receptor blockade reduces mortality and morbidity in heart failure. Low dosages of spironolactone and eplerenone devoid of substantial diuretic effects have been used in the large randomised clinical trials. Experimental evidence supports the concept that direct actions of MR blockade in the heart and vasculature mediate at least part of the beneficial effects on left ventricular remodelling.

Selected References

THE PROXIMATE CAUSE OF HEART FAILURE: MODELS, REMODELING, AND RE-REMODELING

Definition and Proximate Cause of Heart failure:

Heart failure is a set of signs and symptoms—a syndrome—produced by a pathological interaction among the heart, blood vessels, and their neuroendocrine control. A failing heart is characterized by: (1) reduced rate of cycling of heavy meromyosin heads (the fundamental units of contraction), (2) reduced rate of hydrolysis of ATP to ADP and release of energy, (3) reduced slope (Emax) of the connection of end-systolic points of pressure-volume loops obtained at varying preloads, and (4) decreased force or rate of force development for a given preload. Heart failure has multiple potential causes including valvular and/or myocardial disease, infection or toxicity, but most possess in common an overload of pressure and/or volume which may alter calcium kinetics (release and binding), depolarization and/or repolarization, energy transformation and/or availability, or alignment of contractile elements. The pathway of calcium kinetics is as follows:

(1) Abnormal release of calcium from the sarcoplasmic reticulum through the ryanodine release channel.

(2) The abnormal release is due to depletion of calcium in the sarcoplasmic reticulum due to calcium leaks in diastole, or to abnormal function of the ryanodine release channel.

(3) The deficiency of sarcoplasmic calcium is due to faulty entry into the sarcoplasmic reticulum through the SERCa$^{++}$/ATPase pump and abnormal exit of calcium from the cytosol via the up-regulated Na$^+$/Ca$^{++}$ exchanger.

Deficiency of ATP is not the proximate cause of heart failure, rather there is inadequate shuttling of ATP to the contractile elements.

Models of heart failure:

Models of heart failure are developed to study the genesis of signs and/or symptoms (i.e., pathophysiology), and to investigate therapeutic interventions. Models are classified by etiology: genetic modification (e.g., tropomodulin over-expression), toxic (e.g., cobalt, saponin), infectious (e.g., Trypanosoma cruzi), ischemic (e.g., coronary embolization), electrophysiological (e.g., heart block, rapid pacing), electrical ablation (e.g., transventricular shock), drug-induced (e.g., doxorubicin), pressure overload (e.g., aortic/ pulmonic stenosis), volume overload (e.g., AV shunt, mitral/aortic regurgitation).

The vast majority of dogs presented with signs of heart disease are affected by mitral regurgitation, and are small breed, chondrodystrophic, aged dogs which may have multiple concurrent degenerative diseases (e.g., pulmonary fibrosis, chronic bronchitis, microscopic intramural...
myocardial infarction, chronic interstitial nephritis, lenticular sclerosis, periodontitis). The “Perfect Model” should be produced rapidly, inexpensively, have a high yield, mimic the natural state, safe for the investigator, uniform in severity, controllable, stable, humane; and be acceptable by the academic community, pharmaceutical industry, and drug regulatory agencies. Although acute rupture of either the mitral valves or chordae tendinae mimics acute mitral regurgitation, the disease as observed in the clinic is mimicked better by adding rapid-pacing induced heart failure. The ultimate value of a model is determined by its predictive value (sensitivity and specificity) for providing scientific knowledge which can be extrapolated to the target species. The ultimate value of a model is determined by its predictive value (sensitivity and specificity) for providing scientific knowledge which can be extrapolated to the target species.

**Remodeling:**

Remodeling is a “catch-all” term for structural, biochemical, electrophysiological, physico-chemical, and genetic changes that occur in an organism that alters the quality and/or duration of life. Adaptive (physiologic) remodeling translates to increased quality and or duration of life whereas maladaptive (pathologic) remodeling decreases quality and/or duration of life. Mal-adaptive remodeling often arises from chronic hemodynamic loading and/or neurohumoral activation. Examples of adaptive remodeling are the physiologic responses to exercise (athletes) or that which occurs with pregnancy. Adaptive remodeling is characterized by vascular dilatation, a brief increase in activation of renin-angiotensin-sympathetic nervous activity, and lengthening of action potential duration. Adaptive remodeling may transition from a compensatory, physiologic process to a maladaptive one.

**Reverse remodeling:**

Reverse remodeling, “re-remodeling”, refers to the return towards normal cardiac structure or function. Whereas re-remodeling may occur spontaneously after resolution (e.g., viral myocarditis), withdrawal (e.g., cisapride) or correction (e.g., pulmonic stenosis) of etiological agents, it occurs most frequently in response to active therapy (e.g., surgical closure of patent ductus arteriosus, ventricular assist device, cardiac resynchronization, stem cell transplantation, combination of ACE inhibitor and spironolactone). Re-remodeling is assessed using many of the same indices by which remodeling is determined and quantified: ventricular volumes, ejection fraction, sphericity index, myocardial dysynchrony, arrhythmias, and neuroendocrine activation. Agents known to promote re-remodeling are: ACE inhibitors, aldosterone inhibitors, AT1-antagonists, β-blockers, blockers of sarcolemma Na+/H+ exchanger, and possibly dobutamine. Studies from one laboratory suggested that ACE inhibitor-increase in bradykinin in the left ventricle actually accelerated matrix loss and therefore was ineffective in treating rats with volume overload due to AV shunts. However the same group found that β-receptor blockers decreased activation of the renin-angiotensin system, and that this attenuated Ang II-mediated NE and Epi release into the heart and circulation. Decreasing left ventricular angiotensin-converting enzyme expression in the early phases of volume overload was in turn protective. Therefore they believe that angiotensin-II is important in the remodeling process and should be attenuated pharmacologically, but imply that ACE inhibition did not attenuate angiotensin-II production or activity, or possibly that, contrary to the putative beneficial vasodilatory activity of bradykinin, the increase in bradykinin counteracts the benefits of decreased angiotensin-II production or activity. They also showed that β-receptor blockers protected ventricular function in dogs with iatrogenic mitral regurgitation, supporting the well-known beneficial effect of β-receptor blockers. Since angiotensin-II promotes release and decrease reuptake of NE, it is inexplicable why ACE inhibitors which decrease angiotensin-II should not produce a similar benefit.
THE PROXIMATE CAUSE OF HEART FAILURE: MODELS, REMODELING, AND RE-REMODELING

References


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Bertram Pitt is Professor of Medicine Emeritus at the University of Michigan School of Medicine. He is diplomate of the American Board of Internal Medicine and of the American Board of Cardiology.

He is a member of several professional societies such as the American College of Cardiology, the American Society for Clinical Investigation, the American Physiological Society – Circulation Group, the American Federation for Clinical Research and the American Heart Association. He has received awards like the Forest Dewey Dodrill Award for Excellence in 2001 and the James B. Herrick Award in 2005 (both from the American Heart Association).

He has published more than 500 papers in the most important peer-reviewed journals dealing with cardiovascular diseases (Circulation, American Heart Journal, Journal of the American College of Cardiology, Hypertension, European Heart Journal...). He is the first author of RALES and EPHESUS and he is currently leading large scale trials investigating the clinical benefit of mineralocorticoid receptor blockade in cardiac patients.

Mineralocorticoid receptor blockade (MRB) is emerging as an important component of the therapeutic approach to heart failure (HF). The results of the RALES trial showing a 30% reduction in all cause mortality in patients with chronic severe HF (NYHA class III-IV) due to systolic left ventricular dysfunction (SLVD) randomized to the MRB spironolactone supported by the results of the EPHESUS trial in patients with HF and SLVD post myocardial infarction randomized to the MRB eplerenone has led to a class 1 indication for MRB in both European and US guidelines. The benefits of MRB on mortality in HF appear to be in addition to standard therapy including an angiotensin converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker (ARB), a beta adrenergic receptor blocker (BB), a loop diuretic and digoxin.

Spironolactone at 12.5-50 mg/day has been shown to be effective in reducing mortality in the RALES trial. This beneficial effect has been shown to be related to a reduction in myocardial and vascular inflammation, fibrosis, and hypertrophy as well as to an improvement in nitric oxide availability. The effectiveness of MRB in HF can in part be attributed to the finding that MR expression is increased in HF. Activation of the MR, whether by aldosterone or cortisol, has been shown to result in an up regulation of myocardial calcium channel expression, electrical remodeling of the myocardium, and a propensity toward sudden cardiac death as well ventricular remodeling and death due to progressive HF.

Elevated levels of both plasma aldosterone and cortisol have been shown to predict an increase in mortality and morbidity in patients with chronic HF. Of interest is the recent finding that plasma aldosterone levels in the upper tertile, but within normal limits, are predictive of an increase in mortality and morbidity in patients post myocardial infarction independent of the presence of HF, suggesting an important role of MRB in patients post myocardial infarction without HF or SLVD.

MRB is currently being evaluated in patients with NYHA Class II HF due to SLVD in the EMPHASIS-HF trial and in patients with HF and a normal left ventricular ejection fraction (HFNEF) (Diastolic HF) in the TOPCAT trial. In view of the finding that MRB prevents myocardial and vascular fibrosis and hypertrophy it can be postulated that in the future MRB will play an even more important role in the prevention of HF in patients with essential hypertension and in the treatment of the entire spectrum of HF.
References


**EFFICACY OF SPIRONOLACTONE IN DOGS WITH NATURALLY OCCURRING MYXOMATOUS MITRAL VALVE DISEASE**

1. **Introduction**

   In the RALES study (Randomized ALdactone Evaluation Study) a 31% reduction in the risk of mortality (cardiac causes) in patients receiving Spironolactone led to the premature termination of this study for ethical reasons. Aldosterone is thought to be similarly involved in the pathophysiology of canine heart failure (HF), hence clinical field studies were conducted to test whether Spironolactone would also be beneficial in dogs. The aim of these trials was to assess the effect of Spironolactone therapy on the risk of sudden cardiac death, euthanasia for cardiac reasons, or worsening of HF when compared to placebo in dogs with moderate to severe mitral regurgitation (MR) caused by MMVD (myxomatous mitral valve disease).

2. **Materials and Methods**

   **Animals**

   221 dogs were enrolled from 32 practices in France, Germany, Belgium and Italy, between February 2003 and March 2005. The study was completed in May 2006.

   **Study design**

   This multicenter study was a prospective, double-blinded, placebo-controlled, randomized study. The complete clinical trial process was conducted according to Good Clinical Practice (GCP). Owner consent was obtained with the option to withdraw the dog at any time.

   The follow-up comprised two consecutive stages. At the first stage, dogs were recruited in 2 separate studies: one 2-month study, where Furosemide treatment was mandatory at inclusion, and one 3-month study, where Furosemide was not allowed at the time of inclusion.

   The second stage was a 12-month study involving dogs that had completed either of the first studies. Dogs which completed the first studies were entered into the 12-month study, where they continued to receive the same trial treatment.

   **Inclusion criteria**

   At initiation of the first stage, dogs of any breed or gender were enrolled when they presented with moderate to severe MR caused by MMVD (ISACHC class II and class III). Included dogs with dilated cardiomyopathy (DCM), were excluded from analyses (see results section). Dogs must furthermore have presented at least 3 of the following clinical signs, including at least one of cough, dyspnoea, syncope and at least one of reduced activity, reduced mobility, altered demeanor.

   All dogs were receiving an ACEI (angiotensin converting enzyme inhibitor). Furosemide therapy was prohibited at inclusion for the 3-month study and mandatory at inclusion for the 2-month study.
All dogs which had completed one of the initial two studies could be enrolled in the 12-month study, if agreed by the owner.

**Exclusion criteria**

Excluded dogs were those receiving cardiac medications other than ACEI, Furosemide, Digoxin and L-carnitine, those with acute pulmonary edema, a congenital cardiac disease or a life threatening arrhythmia, or any other diagnosed medical condition or those treated with any drugs which could interact with the assessment of the tested product efficacy (e.g. β-blockers, calcium channels inhibitors). In absence of safety data, pregnant females were not enrolled.

**Randomization and blinding conditions**

Double-blinding conditions were maintained throughout the two stages of follow-up for investigators, owners and study monitors. Dogs kept the same study number throughout studies and so were maintained in the same treatment group: no re-allocation was carried out.

**Treatment**

All dogs received conventional therapy including at least an ACEI. Furosemide was allowed after D5 in the 3-month study and from at least one day before inclusion in the 2-month study. During the follow-up, veterinarians could change the dose rate, initiate or terminate Furosemide treatment. Therapy with Digoxin and/or L-carnitine was also allowed. In addition, dogs received either Spironolactone (2 mg/kg once a day with food) or a placebo.

**Evaluation schedule**

In both first studies, examinations had a similar schedule. Full clinical examination, thoracic radiography, electrocardiography, echocardiography, urine and blood samples were performed on Day 0. Clinical and radiographic examinations and blood sampling were performed during the first week, at Day 28 (except radiographs), Day 56 and Day 84. In the long term study, clinical and radiographic examinations and blood sampling were performed at 3-month intervals. Echocardiographic exams were undertaken at inclusion and after 6 and 12 months.

**Clinical evaluation**

The protocol included assessment of the clinical variables cough, dyspnea, exercise intolerance (outside mobility, activity at home, attitude at the veterinary practice) and syncope. Lateral and dorsal thoracic radiographs were used to evaluate the heart size (using the Buchanan Vertebral Heart Scale) and the presence of pulmonary edema. Standard echocardiography measurements were performed. Classification of the stage of heart failure at inclusion was assessed according to the International Small Animal Cardiac Health Council classification (ISACHC)².

**Survival evaluation**

The primary end-point was cardiac related death, euthanasia due to MR, or severe worsening of MR, which was defined as the need to introduce an unauthorized cardiac therapy or to increase the dose of Furosemide over 10 mg/kg/day to prevent life threatening CHF (congestive heart failure). Where the dog died spontaneously or was euthanized, the investigator specified whether the cause of death was cardiac or non-cardiac and noted the precise reason. Cardiac mortality was assessed by pooling the natural deaths and euthanasia owing to cardiac causes.

**Statistical analyses**

All analyses were two-tailed. The descriptive analysis and initial comparability between treatment groups were made on the characteristics and clinical criteria recorded at inclusion in the two first studies. The baseline data were compared between treatment groups, to check that owner withdrawals had not produced bias, and also between these two studies (study effect). The percentage of morbidity-mortality or mortality events at the end of follow-up was compared between groups using Fisher’s exact test. Survival analysis was performed by log rank test, for comparing the survival of the two treatment groups, and by a multistatorial Cox proportional hazard model to assess the impact of some covariates. The hazard ratio and its 95% confidence interval were also evaluated. Level of significance was set at p<0.05. Values were reported as mean ± standard deviation.

**Overall outcome**

**Effect of therapy on outcome**

In the Spironolactone and control groups respectively, 34.3% and 40% of dogs completed the 15-month period. In the Spironolactone group, 10.8% of dogs reached the primary end-point and 25.5% in the control group (Fisher’s exact test, p=0.0046). Causes of withdrawals not related to HF included the owner’s wish to stop after the first studies, concomitant disease, the owners moved away and a car accident.

The estimated 15 months survival rate was 84% for the Spironolactone dogs and 68% for the control dogs (Log rank test, p=0.017). If only mortality (cardiac death and euthanasia related to MR) is considered, the 15 months survival rate was 92% in the Spironolactone group and 73% in the control group (Log rank test, p=0.0071).

**Cox Proportional Hazard models**

The hazard ratio of treatment effect was 0.45 (95% confidence limits (CLI) 0.22-0.90), p=0.023). This represents a 55% reduction in the risk of morbidity-mortality (=evere degradation, natural death, or euthanasia related to MR). If only mortality (natural death and euthanasia related to MRI) is considered the hazard ratio was 0.31 (95% CI 0.13-0.78, p=0.011) which represented a significant 69% reduction in the risk of mortality.

The study of origin (2-month study or 3-month study) and the duration of cardiac treatment before Day 0 did not affect the estimate of the hazard ratio of the treatment effect, its
The number of patients reaching the endpoints for cardiac reasons (severe deterioration and morbidity-mortality) is 66% in our control group class II. Hence, the estimated survival rate of MMVD-cases starting the study at ISACHC that the included population consisted of 89.6% disease at study inclusion. Considering the reduction in the risk of mortality from cardiac causes, the results in dogs were even more marked with a 69% reduction at 15 months (compared to the 31% reduction observed at 3 years in the RALE Study in human medicine).

The beneficial effects of Spironolactone on survival may not only be explained by the diuretic effect of the drug. In rats 1-3, 10-12 and human 4-5, patients aldosterone induces myocardial and perivascular fibrosis and alters the endothelial function of vessels. 6-11 Studies performed in human patients with CHF showed that these effects are counteracted by aldosterone antagonists 11,12. Recent literature demonstrated that a proportion of dogs with naturally occurring MMVD had intramyocardial arterial changes which were associated with areas of fibrosis in the myocardium, so called replacement fibrosis. 4 The same authors suggest that more severe intramyocardial arteriosclerotic changes and more severe replacement fibrosis shorten the survival time from the onset of cardiac therapy to cardiac death or euthanasia 14. We believe that the observed beneficial effect of Spironolactone on survival time in the present study could partly be related to a counteractive effect of Spironolactone on the arterial changes and the replacement fibrosis.

the small size of the DCM population did not permit a dedicated analysis. The benefit of Spironolactone in this pathology should be more deeply investigated.

In conclusion, this clinical trial concerning Spironolactone therapy over a 15-month period in dogs with moderate to severe MR caused by MMVD demonstrated a beneficial effect of Spironolactone when added to conventional therapy. The risk of cardiac related death/euthanasia was reduced by 69% as compared to conventional therapy alone (i.e., ACE plus Furosemide or Digoxin, if needed). This finding supports Spironolactone as part of the treatment protocol in dogs with MMVD.

References


Endnotes

PRINCIPLES OF SURVIVAL ANALYSIS ILLUSTRATED BY CEVA’S SPIRONOLACTONE TRIALS

Time to event data

In health care research we often measure the time which elapses until some event occurs. We call such data time to event data. Sometimes the event is adverse, such as death, sometimes it is beneficial, such as healing. Because of the examples of time to event data which were first studied, such data are often known as survival or failure time data. The terminal event, death, healing, etc. is called the endpoint. The statistical techniques developed to deal with them are known collectively as survival analysis.

The analysis of time to event data would not require any special methods if we knew the time to event of every subject. What makes time to event data difficult to analyse is that often we do not know the exact survival times of all cases. Some subjects will still be surviving when we want to analyse the data. For some events, such as conception or readmission to hospital, the event may never happen for some subjects. Furthermore, when participants have entered the study at different times, some of the recent entrants may be surviving, but have been observed for a short time only. Their observed survival time may be less than those participants admitted early in the study and who have since experienced the event. When we know for some subjects only that the time to the event is greater than some value, we say that the data are censored. This also known as being withdrawn from follow-up.

Here we shall use the example of the CEVA Santé Animale trials of Spironolactone for the treatment of heart failure in dogs. This was a double-blind randomised trial comparing Spironolactone against placebo (the Control group). The event was death or euthanasia because of heart failure and the time to death or euthanasia was recorded in days. Censoring occurred because some animals had not died and were still alive at the time of analysis, because some dogs were withdrawn from trial at owners’ request, usually because they no longer wished to have the regular assessments of the dog, and because some dogs died from other causes, such as road traffic accidents. Data are for 15 months of follow-up.

Kaplan Meier survival estimates

Some censored times may be shorter than some times to events. We overcome this difficulty by the construction of what we call a life table. We follow a hypothetical cohort from any time point onwards. I shall show how this works through the example.
The start of the calculation is set out in Table 2. For each time when an event or censoring occurs, we find the number of dogs who were present at that time, called the number at risk. There were 110 dogs at the start. We find the number who died. For the first time and the second time, we find the number surviving to the end of day 6. On day 7 there is a censoring. Thus, in the first column, the first time was 6 days and the dog died. The second time was 7 days and the dog was censored.

The survival estimate is only an estimate, and if we were to repeat the trial with a new sample of dogs we would get a different survival curve. To allow for this uncertainty, we can add a 95% confidence interval for the survival estimate. This gives what are called Greenwood bounds around the survival curve. This gives a range within which we estimate the survival for all dogs in the population to be. Figure 3 shows these for the Control group.

The Kaplan Meier survival curve

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Figure 1. Kaplan Meier survival curve for the Control group.

Figure 2. Kaplan Meier survival curves for the Spironolactone and Control groups

The survival estimate is only an estimate, and if we were to repeat the trial with a new sample of dogs we would get a different survival curve. To allow for this uncertainty, we can add a 95% confidence interval for the survival estimate. This gives what are called Greenwood bounds around the survival curve. This gives a range within which we estimate the survival for all dogs in the population to be. Figure 3 shows these for the Control group.
The survival curve shows the estimated proportion surviving at any chosen time, but we can also estimate the time to which any chosen proportion of dogs will survive. For example, we might want to find the median survival time, the time to which half the dogs survive. We draw a horizontal line through the chosen proportion, 0.50, to intersect the survival curve (Figure 4).

Figure 4. Attempt to estimate the median survival time for the Control group

We can find the 75% survival time, when 25% of the dogs have died, as shown in Figure 5. We draw vertically down to the time axis to estimate the 75% survival time as 335 days.

Figure 5. Estimation of the 75% survival time for the Control group

However, the survival curve and the horizontal line do not meet. We cannot estimate the median survival time, because too few dogs have died at the time of follow-up.

We can find the 95% confidence interval for this estimate by drawing downwards from where our 75% line intersects the Greenwood bounds, as shown in Figure 6.

Figure 6. Confidence interval for the 75% survival time for the Control group

But it is not a precise estimate. The lower limit of the 95% confidence interval is 154 days. However, the upper limit does not exist. The 0.75 survival line does not cross the upper Greenwood bound. Too few dogs have died for us to produce a meaningful estimate.

The logrank test

We want to be able to compare the survival in the treatment groups, to say whether there is good evidence for a real difference between the treatments (that is, whether the difference is statistically significant) and to estimate how big that difference is.

Greenwood standard errors and confidence intervals for the survival probabilities are useful for estimates such as the one year survival rate. They are not a good method for comparing survival curves. They do not include all the data and the comparison would depend on the time chosen.

Instead of the Greenwood method, to compare survival curves we need a method which makes use of the full survival data. There are several significance tests which we can use for this, of which the best known is the logrank test. This is a non-parametric test which makes use of the full survival data without making any assumption about the shape of the survival curve.

The logrank test tests the null hypothesis that, at any time, the chance of a member of a group experiencing an event is the same for both groups, though the actual chance of an event may change over time. The alternative hypothesis is that at some time the chance of an event is different in the two groups, which would make the survival curves different.

We calculate how many of the 28 deaths observed would be expected to be in the Spironolactone group and how many would be expected in the control group, if there were no difference between Spironolactone and placebo. The precise number expected depends on the pattern of censoring in the groups. On each day where there are deaths, for each group we calculate the total number of deaths for the day divided by the number in the group. Thus we split the number of deaths between the two groups in proportion to the numbers at risk. We then add these for each group. For the trial data, we would expect 13.11 deaths in the Spironolactone group and 14.89 deaths in the Control group. We actually observed 6 deaths in the Spironolactone group and 22 deaths in the Control group. The probability of a difference this large between what we observe and what we would expect is 0.007. The difference is statistically highly significant.

The hazard ratio

To produce an estimate of the size of the difference in survival, we have to make some assumptions about the shape of the curve. We have to assume that they are similar in some way, so that we can find some numerical value to compare between them. We can use the Greenwood standard errors to find a confidence interval for the difference between the survival probabilities at a given time, but this does not use all the data, events after the chosen time being ignored. The best way to estimate the difference between the survival curves uses the hazard, which is a measure of the chance that a member of the population will have an event at any given time.
or the rate at which events happen. To be more precise, we find the probability of an event in any small time interval by multiplying the width of the time interval by the hazard at that time. Hazard depends on the survival time, so that it might increase or decrease as follow-up goes on. If we can assume that the survival curves in the two treatment groups follow the same pattern, then we can assume that if hazard is greater in one group than in the other at one time, it will also be greater at another time. If this is the case, we assume that the hazard in one group is equal to the hazard in the other group multiplied by a constant number, which we will estimate. Thus, if members of one group have twice the risk of an event for members of the other group on the first day, they will also have twice the risk of the event on the second day, twice the risk on the third day, and so on.

The constant ratio is called the hazard ratio. If the risk of an event is the same in the two groups, the hazard ratio is equal to one. If the risk is lower in the intervention group than in the control group, the hazard ratio is less than one. If the risk is greater in the intervention group, the hazard ratio is greater than one. For the hazard in the Spironolactone group divided by the hazard in the Control group, the hazard ratio is 0.31 with 95% confidence interval 0.13 to 0.76. At any given time, the risk of death in the Spironolactone group is estimated to be one third of that in the Control group.

We can adjust the treatment hazard ratio for other variables which may influence survival, using a method called Cox proportional hazards regression. One obvious variable to adjust for is study of origin, the dogs having originally been in two different medium-term studies. These were then combined to produce the long-term study.

The adjustment has no discernable effect, the hazard ratio and confidence interval being unchanged to two decimal places.

Other variables were used in the earlier analysis as potential predictors of survival: the duration of cardiac therapy before inclusion, and concomitant treatment with furosemide. In Cox regression, we usually limit the number of variables to at most one per 10 events. We have 28 deaths among the dogs, so here the maximum number of predictors for a reliable analysis is three. We can add each of these variables to the regression on treatment and original study, separately. When we do this, there is virtually no effect on survival and the hazard ratio for treatment with Spironolactone remains almost unchanged.

Presenting the results of survival analysis

The hazard ratio is the usual statistic used to report the results of survival analysis in the medical literature, where such analyses are much more common than in the veterinary literature. For example, we can see this in major medical journals. The *Lancet* is one of the most highly cited international medical journals and reports many studies using survival data. In the first three months of 2007, there were 13 papers which reported the results of survival data in the Summary. Of these 13 papers, 12 quoted hazard ratios or statistics derived directly from them, such as percentage reduction in the mortality rate, 7 quoted Kaplan Meier survival proportion estimates, and none quoted median survival time estimates or any other estimates of survival times.

Of these 13 papers, 10 reported randomised trials. The main hazard ratios for these trials were: 0.40, 0.47, 0.64, 0.66, 0.69, 0.76, 0.78, 0.81, 0.83, 0.97. (Where hazard ratios were greater than 1.0, I have reversed the order of treatments to make them comparable to the Spironolactone trial). All of these were closer to 1.0 (no effect) than the 0.31 observed for Spironolactone and the mean hazard ratio = 0.70. Hence this trial has produced an unusually large effect.

Survival analysis has proved so valuable that Kaplan and Meier (1958) and Cox (1972) are the two mostly highly cited statistical papers to date (Ryan and Woodall, 2005).

References


CURRENT CLINICAL STATUS AND FUTURE DIRECTIONS FOR BIOMARKERS IN HEART FAILURE

Introduction:

Biomarkers provide the clinical cardiologist with a number of laboratory tests for defining the molecular diagnosis, assessing new risk factors, and better targeting the pharmaceutical approaches in patients with cardiovascular disease. An increasing number of novel risk factors have been added to the classical risk factors of cardiovascular disease. The recent surge of genetic analysis procedures will likely soon.

This review is focused on the current clinical status and future directions for biomarkers in heart failure. All major biomarkers currently used in clinical practice, and other candidate biomarkers are listed in Table 1, from an excellent overview.

Overview of Current Knowledge

Natriuretic Peptides

Three peptides comprise the natriuretic peptide family, including atrial natriuretic peptide (ANP), BNP, and C-type natriuretic peptide. Elevations of these peptides have been associated with varying degrees of cardiac impairment. Two forms of natriuretic peptide, BNP (B-Type Natriuretic Peptide) and its precursor, NT-Pro BNP have been studied as aids to establish the diagnosis, estimate prognosis and monitor the response to therapy of patients with ADHF. Recent studies have established their diagnostic value in patients presenting with dyspnea, their association with prognosis and early results suggest their potential role in assessing response to therapy in ADHF.

Natriuretic peptides are attractive biomarkers in HF for several reasons. An ideal biomarker should possess a high degree of sensitivity and specificity for the disease state which has been demonstrated for natriuretic peptides in HF. Their enhanced gene expression is evident in early HF, and plasma levels increase as HF progresses. Natriuretic peptides are closely linked to the pathophysiology of HF, as their actions include diuretic, natriuretic, vasodilatory, and anti-fibrotic effects, and they suppress renin angiotensin aldosterone system activity. The biologic responsiveness to these physiologic actions is blunted in patients with HF. Multiple hypotheses have been proposed to explain this observed resistance to the biologic effects of natriuretic peptides. Numerous clinical studies described below point to their clinical utility in HF.

Diagnostic Utility

The diagnostic value of BNP was evaluated in the Breathing Not Properly study. BNP levels were significantly higher in patients with dyspnea due to congestive heart failure. McCullough et al reported the diagnostic accuracy for physician clinical judgment, BNP and both. The diagnostic accuracy associated with BNP levels >100 pg/mL was B1%,
compared with 74% for clinical judgment alone. Combining BNP with clinical judgment resulted in an area under the ROC curve of 0.93. In the PRIDE study, NT-Pro BNP results were correlated with a clinical diagnosis of acute HF. NT-Pro BNP levels increase with age so that the study investigators recommended cut points of > 450 pg/ml for patients younger than 50 years of age and > 900 pg/ml for patients age 50 years or older. These cut points were associated with highly sensitive and specific for HF in this study.

**Prognostic Utility**

Serial measurements of BNP appear to provide prognostic information beyond that achieved with a single baseline determination. Patients whose BNP increased from baseline to 4 months had a higher mortality risk as compared to patients whose BNP stays below the median throughout the study. Increasing BNP was also associated with increases in left ventricular end-diastolic diameter, whereas decreasing BNP was associated with decreases in this measure. These data suggest that monitoring BNP change during follow-up may aid in patient risk stratification.

**Challenges Facing Natriuretic Peptides as Biomarkers**

While natriuretic peptides are well validated as biomarkers in HF, several cautions related to the clinical use should be recognized. Consistency in the method used to serially monitor BNP is important. Assay results for BNP may vary widely due to differences in antibody cross-reactivity. In addition, demographics and comorbidities such as female gender, renal impairment, obesity, and atrial fibrillation may significantly influence natriuretic peptide values independent of the degree of HF present. Whereas BNP is an established indicator of decompensated HF, substantial variation in BNP levels have been reported for ambulatory patients with chronic heart failure. As discussed in detail below, several recent reports question whether assays used in clinical practice are actually measuring the biologically active form of natriuretic peptides. These findings indicate that altered forms of BNP contribute to the values detected by various clinical assays. Whether more specific measurement the active form of BNP would be of greater clinical value than existing assays, remains to be determined. Other natriuretic peptide gene products could be useful HF biomarkers, but more research is needed to better identify them and determine their diagnostic and prognostic significance.

**Necrosis Markers**

Cardiac troponins are well established as the markers of choice for myocardial injury and necrosis in acute coronary syndromes. They appear in the plasma rapidly after myocardial injury with a high sensitivity and specificity. Cardiac troponins are detectable even when creatine kinases are not and the troponin T and I isoforms are unique for cardiac tissue. Recent studies have also documented increased circulating troponin in HF patients in the absence of acute ischemia, leading to extensive investigation of this molecule as a biomarker in this syndrome.

**Association of Troponin with Clinical Outcome**

Although elevations are modest compare to those seen in acute coronary syndrome, several studies have demonstrated an association between abnormal troponin levels and subsequent clinical events in HF. Serial measurements of troponin may also have important value. Troponin release during follow-up is associated with a higher mortality risk and a higher risk of heart failure hospitalization. Abnormal troponin release is an attractive biomarker for HF because it seems to reflect loss and/or progressive dysfunction of cardiac myocytes. Troponin may be useful in the selection of patients who may be appropriate candidates for therapies targeting the prevention of myocardial necrosis. Troponins may also be valuable as components of a multimarker strategy to identify high risk patients. Although troponin release seems to reliably identify myocyte injury, it does not indicate a specific mechanism of injury. Further research should provide a better understanding of the mechanism(s) of troponin release and may lead to the identification of other clinically important markers of the underlying pathophysiological process of cardiomyocyte compromise and loss. Elevated troponin is a consistent predictor of mortality in HF. However, additional studies are needed to determine troponin suitability as surrogate marker of response to drug therapy.

**Fibrosis Markers**

Extracardiac matrix (ECM) plays an important role in the support of myocytes and fibroblasts. Collagen is the principal structural protein and collagen types I and III are the most abundant in the myocardium. Collagen type I has a poor specificity but represents the majority of cardiac collagen (85%) and confers tensile strength and resistance to stretch and deformation. Type III is less abundant but more specific to the heart and confers resilience. Fibrafil collagen types within the myocardium are substrates for matrix metalloproteinases (MMPs). Among the MMPs, MMP-1 has the highest affinity for fibrafil collagen and preferentially degrades collagen I and III. The net level of MMP-1 activity is dependent on the relative concentrations of active enzyme and of a family of tissue inhibitors of metalloproteinases (TIMPs). MMP-1 and TIMP-1 are co-expressed in cardiac fibroblasts and are tightly regulated to maintain the architecture of the ECM. MMP-1 is a pyridinoline–cross-linked telopeptide produced as a result of the hydrolysis of collagen type I fibrils by MMP-1 and is a marker of collagen type I degradation. The disruption of the equilibrium between the synthesis and degradation of the ECM results in an excessive accumulation of collagen type I and III fibers within the myocardium. ECM remodeling is an essential process in cardiac remodeling, hypertensive cardiac hypertrophy, dilated cardiomyopathy, and post infarction healing. ECM turnover is influenced by ischemia, stretch, inflammation, and neurohumoral mediators. Myocardial fibrosis is therefore the consequence of a number of pathologic processes mediated by mechanical, neurohumoral, and cytokine factors. Cardiac fibrosis, a major determinant of diastolic dysfunction and pumping capacity, results in tissue heterogeneity and anisotropy, provides the structural substrate for dys-synchrony and arrhythmogenicity, thus potentially contributing to the progression of congestive heart failure (HF) and sudden cardiac death. ECM turnover may be the target of therapeutic agents aimed at preventing or limiting the progression of adverse cardiac remodeling in HF and therefore hospitalization for HF as well as death due to progressive HF and sudden cardiac death. Given the importance of fibrous tissue in the pathophysiology of myocardial dysfunction and failure the non-invasive assessment of fibrosis could prove to be a clinically useful tool, particularly given the potential for cardioprotective and cardio regenerative pharmacological strategies.
The measurement of various serum peptides arising from the metabolism of collagen types I and III may provide information on the extent of myocardial fibrosis and thus prognosis as well as clues to appropriate strategies to improve prognosis. Since procollagen type I C-terminal propeptide (PICP), amino-terminal propeptides of type-I procollagen (PIINP), and N terminal type III collagen peptide (PIIINP) are released with collagen type I or III molecules in a stoichiometric manner during collagen biosynthesis, they are important markers of this process. Although these markers are not specific to the myocardium, studies have shown a correlation between myocardial collagen content and the serum concentration of PICP in patients with hypertension and have demonstrated that serum PICP is secreted by the heart via the coronary sinus in patients with hypertensive heart disease. The PIP/PITP ratio, an index of coupling between the synthesis and degradation of collagen type I, was found to be higher in hypertensive patients with increased collagen accumulation in myocardial tissue than in those with normal collagen accumulation. This evidence linking serum ECM markers to the heart ECM content provides a rationale for their use as biomarkers of ECM remodeling in cardiac disease. MMP-1 and TIMP-1 levels in coronary sinus blood are higher in patients with HF, but not in normotensive subjects, although there was no association between blood pressure and TIMP-1 levels in coronary sinus blood of hypertensive patients with increased collagen accumulation in myocardial tissue than in those with normal collagen accumulation. This evidence linking serum ECM markers to the heart ECM content provides a rationale for their use as biomarkers of ECM remodeling in cardiac disease.

**Multimarker Strategy**

Since several biomarkers appear informative for the diagnosis and prognosis of HF, interest has developed in the potential clinical use of multiple markers simultaneously in individual patients. Combining biomarkers incorporates potentially additive information and may provide a more accurate representation of a patient’s risk profile. Ishii et al studied 98 patients hospitalized for decompensated HF. Troponin T, troponin I, and BNP were obtained at the time of admission. The optimal value of troponin and BNP to predict clinical events was determined by a receiver operating curve approach. Patients were categorized into low, intermediate, and high risk groups according to troponin and BNP levels. As expected, low risk patients had low troponin and low BNP. Intermediate risk patients had either troponin or BNP above the optimal prediction level, while high risk patients had both troponin and BNP above the optimal prediction level. The combination of troponin and BNP provided a more accurate risk stratification than either of the parameters alone. The authors of this study replicated their findings in a separate patient cohort. Similar findings have also been reported by Horwich et al. Patients with elevations of both BNP and troponin had the highest mortality in a cohort of 238 patients with advanced HF undergoing transplant evaluation. Multimarker strategies are attractive because they allow the integration of several components of the pathophysiological process. Combining BNP and troponin values incorporates an assessment of both congestion and myocardial injury. HF is a complex syndrome with multiple pathophysiological processes that occur simultaneously. Adopting a multimarker strategy may yield more accurate diagnostic and prognostic information since it incorporates more than one aspect of the disease process.

**Novel Potential Biomarkers in Heart Failure**

**Cytokines: Interleukin 1B**

Changes in the elaboration of many cytokines have been identified in HF but no specific cytokine has been established as a clinically useful biomarker to date. The search for novel cytokines as biomarkers in HF continues. Abnormally elevated levels of interleukin-1B (IL-1B) have recently been detected in a small study of patients with heart failure, and ANP mRNA expression increased in IL-1B treated myocytes. In addition, IL-1B levels have been associated with symptom severity. In a study of 86 patients with NYHA Class II-IV heart failure, IL-1B levels were significantly higher among patients with NYHA Class IV symptoms as compared to NYHA Class II or III. Another study reported higher levels of IL-1B in patients with HF as compared to normal controls, and IL-1B was also associated with NYHA Class. In contrast in this patient population, IL-B was not associated with functional class, and there was no detectable correlation between IL-6 and IL-1B. White et al recently reported the results of a study in 29 patients with worsening HF who received 72 hours of milrinone or dobutamine therapy. IL-1B was significantly higher as compared to age matched controls on admission while at 30 days, a significant reduction in IL-1B and other markers of inflammation was observed. Studies suggest the potential for IL-1B as a biomarker in HF but additional studies will be required to determine if this cytokine will be of clinical value in predicting outcome or monitoring therapy in HF.

**Other Natriuretic Peptides**

The family of natriuretic peptides consists of several members, but most clinical recommendations focus only on the assessment of BNP and its precursor NT-Pro BNP. Currently, both are considered superior to atrial natriuretic peptide (ANP) for diagnostic and prognostic assessment of patients with HF, even though ANP comprises most of the natriuretic peptides in the circulation. The assessment of ANP is less reproducible, but measurement of ANP’s precursor proANP may overcome the problem of reproducibility, since it...
is significantly more stable in the circulation than the mature peptide. Assessment of other natriuretic peptides may prove valuable. The predictive value of mid-regional proANP, a novel natriuretic peptide was assessed in a cohort of 525 patients with stable HF. Mid-regional proANP was at least as powerful as NT-ProBNP in predicting mortality in these patients. Mid-regional proANP was superior to NT-ProBNP in important subgroups of patients in which NT-ProBNP failed to predict survival. This was particularly evident in patients with mild disease or the obese. Additional studies in other patient populations with HF are needed to fully validate this marker, but these studies provide support for the need to monitor multiple markers. Other natriuretic biomarkers such as mid-regional pro-adrenomedullin may also prove to be useful.

**Novel Biomedomic Approaches**

Recent advances in molecular biology methods promise to transform the current approach to detection and application of biomarkers in heart failure. Specific examples of recent data from these rapidly evolving fields (based on proteomic and transcriptomic methods) illustrate their potential to fundamentally alter our approach to biomarker characterization of HF.

**Potential for biomarkers to guide therapy**

Conceptually, the use of biomarkers to guide therapeutic decisions is attractive and already supported by a variety of observational studies and a few preliminary clinical trials. However, no biomarker to date has been clearly established for this indication in HF. Interest continues to be driven by the lack of precision in early clinical diagnosis and lack of reliable indicators to guide the therapy in established HF. Many HF patients remain at high risk despite advances in therapy. Using classical clinical assessments has not prevented significant treatment gaps in the use of evidence-based medication which persist despite strongly positive results from randomized clinical trials. Observational prognostic studies demonstrate convincing evidence that biomarker measurements provide a more accurate assessment of risk than clinical assessment alone and suggest that knowledge of biomarkers could help optimize therapy in HF.

As a foundation and rationale for larger outcomes studies, preliminary findings are now available from several recent pilot trials on biomarker-guided therapy in HF. These results include findings from The Strategies for Tailoring Advanced Heart Failure Regimens in the Outpatient Setting: Brain Natriuretic Peptide Versus the Clinical Congestion Score (STARBRITE) trial and STARS (Treatment monitoring of systolic cardiac insufficiency), which were designed to evaluate whether a BNP guided approach to managing fluid balance is more effective at preventing death or hospitalization as compared to clinical assessment alone in patients hospitalized with acute decompensated HF. These studies provide substantial support for large-scale outcomes studies of the efficacy of monitoring therapy based on BNP.

Troponin is another candidate for consideration as a marker to guide management of patients with heart failure. Although the mechanisms responsible for troponin release are not well understood, elevated troponin detected by current assays does appear to reflect true cardiac myocyte loss and has been strongly associated with adverse outcomes. These findings suggest troponin could serve as a guide/surrogate marker of therapeutic response in HF patients. The proposed rational concept assumes that a therapeutic regimen associated with increased troponin may have a negative effect on clinical outcomes, whereas treatments associated with normalization of troponin or no troponin release is likely to be associated with favorable outcomes. Whether troponin release is secondary to ischemia, remodeling, or elevated filling pressures, targeting these conditions with adjustments of various therapeutic interventions may result in reduced myocyte loss and improved outcomes.

Despite early favorable data, there are still several important aspects of biomarker guided therapy to carefully consider in testing this strategy: 1) benefit and risk associated with specific treatment adjustments in response to changes in biomarkers; 2) use of single versus multiple markers to guide therapy; 3) extent of change in biomarker(s) necessary to alter therapy; and 4) target HF patient population for guided therapy.


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<td>Brain natriuretic peptide</td>
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References

Who are we?

CEVA Santé Animale is the world’s 9th largest animal health company; we research, develop, produce and market pharmaceutical products and vaccines for companion animals, livestock and poultry. The company is based in Libourne, France and is directly invested in 45 other countries with distributive partners in many more, giving us a truly global dimension.

A results culture:

- Originally a subsidiary of the Sanofi group, the Sanofi Santé Nutrition Animale (SSNA) management conducted the first of three leveraged buy outs to launch in 1999 CEVA Santé Animale.
- In the latest LBO completed during 2007, the CEVA Santé Animale’s management and employees assumed a majority stake in the company with the backing of financial partners Euromezzanine and Natixis.

During our first 10 years of existence, the company’s organic growth has averaged 8.4%, well beyond the industry average. We owe our existence to the farmers, pet owners and veterinarians who support us and as a result we are totally dedicated to providing them with products and services, that protect and improve not only the lives of their animals but the well being of us all, as a global community.

Key figures (Dec 2008):

- Sales: Euro 363m.
- Operating income: 49.4m.
- R&D expenditure: 27.6m (7.7% sales)
- Employees: 2175

Geographic and strategic focus: GLOCAL

CEVA Santé Animale is organised around 3 geographic zones, which correspond to the major regulatory and market-based blocks: Europe, North America and Zone International (Africa/Middle East, Asia/Pacific and Latin America).

Our objective is to direct resources towards developing products and services that correspond to the ever increasing specific requirements of local markets.

In turn the corporate marketing groups, working from head office, have global strategic responsibility across 3 target animal groups: companion animals, livestock and poultry.

Each group focuses its activity on the management of strategic products, further defined according to our areas of expertise:

- Livestock: Anti-infectives, Fertility management, Vaccines.
- Poultry: Pharmaceutical products, Vaccines and Equipment.

Innovation is a frequently used word in the pharmaceutical world often associated with the introduction of new “blockbuster” products. CEVA’s approach to innovation, aside from our R&D programme, is to encourage our local and central teams to find better solutions to existing and emerging diseases, which will in turn improve animal health and productivity.

That’s what we mean when we use the word GLOCAL: our aim is to be global in our ambition to tackle the big issues and local in the way that we tailor practical solutions for our customers.

The bigger picture: 75% of emerging human diseases are of animal origin (source: OIE)

CEVA is heavily involved in the fight to protect against diseases such as avian influenza and brucellosis that also directly menace the human population. Our strength and commitment in addressing these diseases goes far beyond our relative market position, this is equally true of our commitment to the emerging markets of the world, where we have invested significantly despite the level of risk.

We believe that a “one health, one planet” approach is required to properly address global animal health issues and we will continue to invest our time and resource to ensure that we make real impact.
Prolong Life!*  

*in combination with conventional therapy.

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