2nd Human and Veterinary Crosstalk Symposium on Aldosterone

October 2011
2nd Human and Veterinary Crosstalk Symposium on Aldosterone

For several years, the ‘One Health’ concept has sought to address health from a more global point of view, because of the close links between human and animal health and the environment. Ceva Santé Animale is committed to this collaborative approach, applied here to cardiology, one of its sectors of expertise. The first joint cardiology symposium organized by Ceva Santé Animale two years ago in Bordeaux was a big success among experts in both human and veterinary cardiology. The scientists particularly appreciated the chance to pool their results and study similarities and differences between the human and canine contexts. The second joint human and veterinary cardiology symposium is a logical follow-up of the First one, and took place over the weekend of 1st and 2nd October 2011. Over one hundred world experts in human and veterinary cardiology were welcomed to Bordeaux. The specialists spent all day Saturday discussing the latest research. Each theme was addressed in turn from the human and veterinary medicine viewpoints, and the rich discussions brought up plenty of ideas on both sides.

This report contains the proceedings of each presentation and a summary of the discussions that came out during each session. We hope you will enjoy reading it.

Sylvie Bourrelier
DVM, Operational Director WE
Companion Animal
Ceva Sante Animale

Emilie Guillot
DVM, Technical Manager Cardiology
Ceva Santé Animale

Speakers at the meeting, from left to right:
Bertram Pitt, Jonathan Elliott, Jens Häggström, Claudio Ronco, Adriaan Voors, Allan Struthers, Frédéric Jaisser, Adrian Boswood, Clarke Atkins, Mark Oyama, Rebecca Stepieen, Michele Borgarelli and Faiez Zannad.
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Allan D. Struthers

BSc, MD, FRCP, FESC, FRSE, FMedSci
Professor of Cardiovascular Medicine
Division of Medical Sciences
University of Dundee,
Centre for Cardiovascular & Lung Biology
Mail Box 2,
Ninewells Hospital & Medical School
Dundee, UK.
Contact: a.d.struthers@dundee.ac.uk

Dr. Allan Struthers graduated MB (Hons) from Glasgow University in 1977. After junior hospital posts in Glasgow, he was Senior Registrar at the Royal Postgraduate Medical School and Hammersmith Hospital in London in 1982-1985. He was then appointed Wellcome Senior Lecturer/Consultant Physician in Dundee and is currently Professor of Cardiovascular Medicine and runs the Heart Failure service at Ninewells Hospital in Dundee.

He was Chairman of the SIGN Guidelines in Heart Failure (2007) and is now Chairman of NHS-QIS Standards (2010) for Heart Failure. In addition, he is also Chairman of Tenovus NSAC and Senior Regional Advisor for SACDA. Professor Struthers runs a large clinical research programme and has supervised over 40 MD/PhDs.

He pioneered the use of plasma BNP to identify heart failure patients and the use of aldosterone blockers to reduce their mortality. Another research interest is in allopurinol and he has recently shown it to have antianginal oxygen sparing effects. He is also using cardiac MRI to assess novel ways to regress left ventricular hypertrophy, e.g. Vitamin D. He is currently funded (as PI) by the British Heart Fundation, Medical Research Council, CSO, Diabetes (UK) and Chest, Heart & Stroke. In total he has held 30 BHF grants.

He has published over 400 papers which are cited around 600 times every year. His research h-factor is high at 51.
Clinical aspects of aldosterone and the «aldosterone-escape» concept: what is the role of aldosterone receptor blockade?

ALDOSTERONE RECEPTOR ANTAGONISTS CLINICAL INTEREST: BEYOND THE “ALDOSTERONE ESCAPE” CONCEPT

Aldosterone escape: background.

ACE inhibitors began to be used in human heart failure in the late 1980s. The main impetus for their use were the CONSENSUS I, SOLVD and SAVE trials.1,2,3 Thereafter it began to be realised that neither angiotensin II nor aldosterone were fully suppressed by ACE inhibitors. The latter phenomenon was called “Aldosterone Escape” whereby the initial decrease in aldosterone produced by an ACE inhibitor was reversed and aldosterone levels returned to normal.

Bomback and Klemmer4 summarize the results of eight studies indicating that aldosterone breakthrough occurs in a significant proportion of patients on long-term ACE inhibitor and/or ARB therapy, and that the phenomenon might be associated with important cardiovascular and renal outcomes, including left ventricular hypertrophy, poor exercise tolerance, refractory proteinuria, and declining glomerular filtration rate.

The phenomenon of aldosterone escape could perhaps have been expected since aldosterone has two principal secretagogues: angiotensin II and potassium. Although ACE inhibitors decrease angiotensin II, they also increase potassium and the latter, as a secretagogue for aldosterone, would be expected to offset the tendency for a low angiotensin II level to produce a low level of aldosterone.

Interest of using aldosterone receptor blockers in Heart Failure (HF).

Consequently, the concept of aldosterone escape led to the exploration of whether adding spironolactone to an ACE inhibitor in human heart failure would produce any benefits.

Barr et al5 showed that spironolactone had beneficial cardiac effects (on sympathetic activity and on ventricular arrhythmias) when added to an ACE inhibitor in human heart failure.

Aldosterone blockade was then shown clearly to reduce total mortality in both the RALES and EPHESUS studies.6,7 In the RALES study, a 30% reduction in all cause mortality was shown in patients with chronic severe HF (NYHA class III-IV) due to systolic dysfunction (SLVD) receiving spironolactone whereas the EPHESUS trial showed that the addition of eplerenone to optimal medical therapy reduces morbidity and mortality among patients with acute myocardial infarction complicated by SLVD and HF.6-8 Aldosterone blockade hence became a mainstay of therapy in human heart failure and was included as mandatory in all heart failure guidelines.
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ALDOSTERONE RECEPTOR ANTAGONISTS CLINICAL INTEREST: BEYOND THE “ALDOSTERONE ESCAPE” CONCEPT

Beyond the aldosterone escape concept.

The question then arose, especially in hypertension, as to whether patients would benefit from aldosterone blockade, whatever their aldosterone plasma levels. Parthasarathy et al.9 showed that spironolactone reduced blood pressure in patients with both high and low plasma levels of aldosterone. It then became clear that aldosterone blockade was beneficial irrespective of the individual’s plasma aldosterone level.

Similarly, Palmer et al.10 demonstrated that plasma aldosterone levels in the upper tertile, but within normal limits, are predictive of an increase in mortality and morbidity in patients with post myocardial infarction independent of the presence of HF, suggesting an important role of aldosterone blockade in patients with post myocardial infarction without HF or SLVD.

In the EMPHASIS-HF trial, conducted in NYHA Class II HF due to SLVD patients, i.e. patients with systolic heart failure and mild symptoms, eplerenone, as compared with placebo, reduced both the risk of death and the risk of hospitalization.11

Hence, the results of the RALES, EPHESUS and EMPHASIS-HF studies allow to conclude that, aldosterone blockade reduces mortality in both mild and severe heart failure.6,7,11

In addition, these studies may help to support that aldosterone blockade is beneficial irrespective of the individual’s plasma aldosterone level.12

Therefore in humans with heart failure due to left ventricular systolic dysfunction, aldosterone blockade is strongly indicated, irrespective of the patient’s aldosterone levels and irrespective of the severity of their heart failure.

References.


Adrian Boswood graduated from Cambridge University Veterinary School in 1989. He has worked at the Royal Veterinary College since joining as an Intern in 1990. He obtained the European College of Veterinary Internal Medicine Cardiology Diploma in 2001 and is a European Specialist in Veterinary Cardiology.

Adrian is Professor of Veterinary Cardiology and his main area of interest is small animal cardiorespiratory medicine. His research interests include the clinical uses of cardiac biomarkers and the treatment of heart disease and failure.
Background.

The renin-angiotensin-aldosterone system (RAAS) is one of the biological systems that is stimulated in patients with heart disease. Increased concentrations of aldosterone are thought to underlie the development of detrimental changes to the myocardium and vasculature, as well as resulting in sodium and water retention. Favourable effects of mineralocorticoid receptor blockade have been seen in human and canine patients with heart disease and heart failure.

Previous studies have evaluated circulating concentrations of aldosterone in canine heart disease patients, but findings have been somewhat contradictory and confounded by the effects of therapy. It has recently been proposed that measurement of urinary aldosterone to creatinine ratio may provide a reliable estimate of 24 hour urinary aldosterone secretion and therefore give an indication of average aldosterone production less subject to the rapid fluctuations of plasma concentrations.

The aims of this study were to measure the urine aldosterone to creatinine ratio (UAC) in a large population of dogs with naturally occurring degenerative mitral valve disease (DMVD) and to investigate the relationship between UAC and clinical variables including echocardiographic measurements.

Materials and Methods.

The dogs included in this study are part of an ongoing longitudinal study of dogs with DMVD which began in 2004. The study is approved by the ethics and welfare committee of the Royal Veterinary College. Dogs were prospectively recruited through two first opinion practices in Central London. In order to be included in the study dogs needed to have demonstrable DMVD and be free of other significant systemic disease at the time of recruitment.

Dogs were examined at approximately six month intervals and at each examination they were weighed, underwent a physical examination and a series of diagnostic tests. Owners were asked to collect a free catch urine sample the evening prior to a patient’s appointment and on the morning of the appointment.

An age and weight matched population of normal dogs was also recruited. These dogs were normal on the basis of a physical examination.
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Echocardiographic examination was carried out on all dogs affected by valvular disease. In some dogs it was carried out on multiple occasions. Standard 2-D, M-mode and Doppler examination was undertaken with patients conscious and restrained in lateral recumbency. This was in order to confirm the diagnosis of the underlying disease process and also to obtain the measurements used in the analysis.

LVEDDN and LVESDN were calculated using the left ventricular internal diameter in diastole, the left ventricular internal diameter in systole and the bodyweight according to the formulae described by Cornell and others. In dogs which were examined three times the second visit was designated as the “baseline” visit. The prior percentage changes in LVEDDN and LVESDN per month were calculated by comparing the measurements from the baseline and previous examinations as follows:

Prior percentage change per month = ((Visit 2 – Visit 1)/Visit 1) * 100)/ time between visit 1 and visit 2 (months).

Similarly, the subsequent percentage changes in LVEDDN and LVESDN were calculated by comparing the measurements from the baseline and subsequent examinations as follows:

Subsequent percentage change per month = ((Visit 3 – Visit 2)/Visit 2) * 100)/ time between visit 2 and visit 3 (months)

If the dog was only examined twice, either the first or the second visit was designated the baseline visit at random. If the first visit was designated the baseline visit, subsequent percentage changes were calculated. If the second visit was designated the baseline visit, prior percentage changes were calculated.

Urine was centrifuged and then the supernatant frozen at -80° centigrade until undergoing analysis. Urinary aldosterone was measured following mild acid hydrolysis and extraction into ethyl acetate. Aldosterone was measured (in pg/mL) using a commercially-available radioimmunoassay, validated for use in dogs. Urine creatinine was measured in (µmol/L) by a commercial laboratory.

Statistical analysis

UAC values were compared between dogs with different classes of DMVD using one way ANOVA.

Univariable and multivariable regression analyses were used to assess associations in dogs with MMVD between UAC and clinical characteristics (age, breed (CKCS: yes/no), body weight, HR obtained from the electrocardiogram, echocardiographic measurements (LA/Ao ratio, LVEDD/ LVFWd ratio, LVEDDN, LVESDN, prior percentage change in LVEDDN and LVESDN per month and subsequent percentage change in LVEDDN and LVESDN per month) and treatment with angiotensin converting enzyme inhibitors (ACEi: yes/no), pimobendan (yes/no) or diuretics (yes/no). CKCS was chosen as the comparator breed since this was the most frequently represented breed, and this breed is particularly prone to MMVD and
previous studies have suggested differences in the natural history of the disease in this breed. Variables associated with UAC with P < 0.2 in the univariable analysis were entered into the multivariable analyses. Separate multivariable analyses were run excluding and then including the percentage change in normalised ventricular dimensions.

In the multivariable regression models, analyses were performed in a backward stepwise manner. All variables were initially included, and the variable with the highest P-value was removed until all remaining variables had a value of P < 0.05.

**Results.**

Numerical results will be presented at the meeting.

In the multivariable model which excluded rate of change data, age was negatively associated with UAC, whereas being a CKCS and receiving diuretics were positively associated with UAC.

In the multivariable model which included rate of change data, age was negatively associated with UAC whereas being a CKCS, prior percentage change in LVEDDN and subsequent percentage change in LVESDN were positively associated with UAC.

**Discussion.**

Our study has demonstrated a number of novel and interesting findings. UAC appears to be higher in CKCS by comparison to other breeds and this effect seems independent of the influence of age and stage of disease. UAC also seems to be higher at times of active ventricular remodelling; showing a significant relationship with prior change in ventricular diastolic diameter and subsequent change in ventricular systolic diameter.

Our findings suggest that aldosterone production, as indicated by the UAC, is increased at times of active ventricular remodelling in dogs with DMVD.

**Abbreviations.**

- **ACEi** angiotensin converting enzyme inhibitor
- **CKCS** cavalier King Charles spaniel
- **HR** heart rate
- **LA/ Ao** ratio of left atrial diameter to aortic root diameter
- **LVEDD/diastolic** ratio of left ventricular end diastolic diameter to aortic root diameter
- **LVFWd** diameter to left ventricular free wall thickness in diastole
- **LVEDDN** left ventricular end-diastolic diameter, normalised for body weight
- **LVESDN** left ventricular end-systolic diameter, normalised for body weight
- **MMVD** myxomatous mitral valve disease
- **RAAS** renin-angiotensin-aldosterone system
- **UAC** urinary aldosterone to creatinine ratio
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References.


Clarke E. Atkins

DVM, DipACVIM  
[Internal medicine and Cardiology]

Jane Lewis Seaks Distinguished  
Professorship of Companion Animal Medicine  
Department of Clinical Sciences  
College of Veterinary Medicine  
North Carolina State University  
Raleigh, North Carolina, USA.  
Contact: ceatkins@ncsu.edu

Clarke E. Atkins, DVM, Professor of Medicine and Cardiology at North Carolina State University is a 1972 graduate of the University of California, Davis and an Angell Memorial Animal Hospital intern. He is board-certified by the ACVIM in internal medicine and in cardiology.

Dr. Atkins is known for his research and teaching in small animal cardiology, he is the 2004 Norden-Award recipient for excellence in teaching, and was recently named the Jane Lewis Seaks Distinguished Professor.

He is the author of over 150 publications and his research involves canine and feline heartworm disease and pharmacologic therapies of cardiac disease in dogs, cats, and horses.
Clinical aspects of aldosterone and the «aldosterone-escape» concept: what is the role of aldosterone receptor blockade?

WHERE ARE WE WITH ALDOSTERONE ESCAPE (BREAK-THROUGH) IN 2011?

Clarke Atkins, DVM, Andrea Lantis, DVM, Marisa Ames, DVM.

Cardiovascular disease in dogs produces significant morbidity and mortality and ranks 2nd in importance, behind only neoplastic disease as a cause of non-traumatic death in dogs. The most important non-parasitic cardiovascular disease affecting dogs is degenerative mitral valvular disease with mitral regurgitation (MR).

An ACVIM consensus panel has unanimously indicated that chronic pharmacologic management of heart failure in dogs caused by MR should include furosemide, pimobendan, and an angiotensin converting-enzyme inhibitor (ACE-I), with the majority of panelists also recommending a mineralocorticoid receptor blocker (MRB), such as spironolactone. The use of the latter 2 drugs (ACE-I and MRB) demonstrates the priority placed upon suppression of the renin-angiotensin-aldosterone system (RAAS) in canine cardiovascular disease. The ACE-I, utilized in veterinary medicine, blunt plasma ACE activity maximally by approximately 75%, when administered as directed, and the benefits of ACE-inhibition have been demonstrated in multiple clinical trials in dogs with CHF due to chronic degenerative valvular disease and dilated cardiomyopathy.

Definition and Incidence.

These above-mentioned benefits are also appreciated in human cardiovascular and renal patients. It is recognized in human heart failure-patients, however, that a percentage of the population experiences a recrudescence of aldosterone and angiotensin II secretion with chronic ACE-inhibition. Persistent aldosterone secretion, despite a significant reduction in plasma ACE activity and presumed removal of angiotensin II, its major secretagogue, is then referred to as “aldosterone escape” or preferably “aldosterone breakthrough”. Aldosterone escape has been defined in the human literature as any increase in serum aldosterone concentration that exceeds a baseline value after initiation of RAAS-blocking therapy. To date, no study has been reported which precisely assesses the time from onset of treatment until breakthrough or the percentage of patients affected. However, a recent meta-analysis revealed varying results, depending the exact definition that aldosterone breakthrough was present in ~10% patients in the first 6 months of ACE-inhibition therapy and in 40-50% after 12 months of therapy (Fig 1). There appears, however, to be no consensus within the human literature regarding the time course of aldosterone escape as some authors...
describe it as a serum level that exceeds a baseline (pre-ACEI and/or ARB therapy) value 6-12 months after initiation of RAAS-blocking therapy"\(^1\) while others have documented aldosterone escape in human patients 4-6 weeks after initiation of an ACEI.\(^9, 12-13\) Some investigators have directly addressed whether the incidence of aldosterone breakthrough was related to the dosage or class of RAAS blockade and found no apparent differences. As examples, Tang et al. found no significant difference in the incidence of breakthrough between groups of patients randomly assigned to low dose or high-dose enalapril and Horita et al. demonstrated equal rates of breakthrough among subjects on ACE inhibitors, ARBs, or a combination.\(^14\)

The frequency, degree and importance of aldosterone breakthrough are not well understood in humans, let alone animals. Nevertheless, several studies have revealed the likelihood of aldosterone breakthrough in veterinary patients and experimental animals.\(^15-17\) Cats with hypertrophic cardio-myopathy receiving ramipril (0.5 mg/kg q24h) experienced 97% suppression of plasma ACE activity at 3, 6, 9, and 12 months, however, plasma aldosterone was not different in cats treated with ramipril compared with those receiving placebo.\(^16\) A pacing-induced CHF model in dogs receiving an intravenous bolus of fosinoprilat (1µmol/kg) and intravenous furosemide (40 mg administered over 20 minute) resulted in a significant increase in plasma aldosterone concentration at 30, 60, 90, and 150 minutes.\(^17\)

In a clinical study of 22 Cavalier King Charles Spaniels with naturally-occurring MR and “early” signs of heart failure (modified NYHA class III), 12 dogs receiving enalapril monotherapy (0.4 mg/kg PO.q12h) had significant reductions in plasma ACE activity at 3 weeks (-83%). Furosemide was subsequently added to the treatment regimen at the time of the 3-week re-evaluation. Significant reductions in plasma ACE activity (-81%) were again noted 6 months after the initial examination. However, while administration of enalapril led to a significant decrease in plasma aldosterone concentration after 3 weeks of treatment, the 6-month plasma aldosterone concentration in dogs receiving enalapril and furosemide were significantly increased as compared to initial and 3-week examination (P<0.05).\(^15\) This demonstrates aldosterone breakthrough occurring with the addition of the known RAAS-activator, furosemide.

Finally, in short-term study of normal hound dogs, carried out in our laboratory, furosemide-induced RAAS activation (maximum mean increase at 7 days of ~300%) was not suppressed with concurrent benazepril administration, even though plasma ACE activity was suppressed by approximately 67% six hours post-administration.\(^a\) These final 2 studies argue for alternative RAAS suppressant strategies, such as addition of MRB and/or ARB, possibly earlier in the course of heart failure than previously considered necessary.

**Escape vs Breakthrough.**

Aldosterone “escape”, the original term for this phenomenon in the cardiology literature, has been largely replaced with the term “Aldosterone breakthrough”. The former term (escape) had been previously coined to describe the phenomenon in which the kidneys “escape” the sodium retaining effects of aldosterone to achieve a sodium balance and stable arterial blood pressure, without severe volume expansion and edema.\(^8\) This is contradistinction to the observation of restoration of aldosterone blood or urine concentrations toward baseline
after being initially decreased during RAAS blockade (with either ACE-I or angiotensin II receptor blockers (ARBs)).

**Mechanism.**

The mechanism(s) by which aldosterone breakthrough occurs are not yet well understood and is probably multi-factorial. The most popular explanation is that alternative pathways and enzymes for conversion of Angiotensin I to Angiotensin II are evoked, including chymase and cathepsin G. Renin plasma concentrations are elevated in the presence of ACE-I therapy with subsequent elevations in AngII concentrations, which may contribute to aldosterone breakthrough. Increase in plasma potassium values has also been suggested as a potential mechanism for aldosterone breakthrough, but the available evidence does not support this hypothesis. Three studies reported that potassium levels did not change during an ACE inhibitor therapy longer than 6 months, regardless of breakthrough status. Finally, endogenous factors, such as corticotrophin, catecholamines, endothelin, prolactin, serotonin, and vasopressin, as well as diuretics, sodium restriction and vasodilators stimulate aldosterone secretion and may therefore contribute to breakthrough. To the authors knowledge, compliance failure has not been evaluated as a contributor to aldosterone breakthrough.

**Clinical Relevance.**

Chronic exposure to high concentrations of aldosterone results in excessive sodium retention with expansion of extracellular volume, favors potassium and magnesium wasting, inhibits myocardial norepinephrine uptake, diminishes heart rate variability, produces cardiac arrhythmias, decreases baroreceptor sensitivity, contributes to endothelial dysfunction and vascular inflammation, and is independently associated with renal, vascular and cardiac remodeling and heart failure. Plasma aldosterone levels at presentation are known to be significantly predictive of mortality after myocardial infarction.

Increasing evidence links aldosterone excess and/or activation of mineralocorticoid receptors to the development and progression of various cardiovascular disease processes in humans. The Randomized Aldactone Evaluation Study (RALES) revealed a 31% reduction in mortality due to cardiac causes in human patients with NYHA class III and IV CHF receiving spironolactone when added to conventional therapy (ACE-I, loop diuretic, and digoxin) as compared to a placebo cohort. Pro-collagen markers decreased in the spironolactone group but did not change in the placebo cohort, indicating the benefit of aldosterone blockade paralleled the reduction of cardiac fibrosis. Serum levels of three pro-collagen markers were independently associated with increased risk of death and the beneficial effects of spironolactone on patient survival were predominantly seen among patients with the highest baseline levels of collagen markers. The EPHESUS study compared eplerenone to placebo, each with standard therapy, in post-infarct patients with diminished left ventricular function, resulting in significant survival benefit (17% reduction in cardiovascular mortality). This benefit was noted early, within 30 days of initiation of therapy. Finally, the EMPHASIS-HF study demonstrated that eplerenone in a population of mild (NYHA II) heart failure patients with left ventricular dysfunction, significantly reduced cardiac death and hospitalization by 29%, as compared to placebo. This study importantly demonstrated benefits of MRB early in the course of heart failure.
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Evidence of the harmful effects of RAAS activation the benefits of RAAS suppression are evident in veterinary patients as well. In a prospective veterinary study by Hezzell et al., urinary aldosterone concentrations were found to be negatively associated with survival (P=0.005) in 54 dogs with mitral valve disease. A recent double-blinded, field study in 212 dogs demonstrated a 69% reduction in risk of cardiac morbidity and mortality in dogs with chronic degenerative mitral valve disease that were treated with spironolactone in addition to an ACEI with furosemide +/- digoxin when compared to furosemide, an ACEI +/- digoxin alone. The therapeutic implications of these studies are that aldosterone breakthrough appears to occur in animals as it does in man and that aldosterone and angiotensin II are important negative prognostic indicators for dogs and humans suffering from cardiac disease and failure. To counteract this, a MRB, such as spironolactone is probably necessary in many heart failure patients and may be necessary earlier than previously thought. The use of renin antagonists may play a future role in treatment of cardiovascular disease in dogs.

Figure 1. The left panel demonstrates 2 definitions of aldosterone breakthrough, with definition 1 defined as a rise above baseline plasma aldosterone concentrations after initial suppression by an angiotensin converting-enzyme inhibitor (ACE-I). The second, more conservative definition, requires that the plasma aldosterone rebound and exceed a certain level (in this example, 80 pg/ml [red line], one of several thresholds chosen by various investigators). The hypothetical patient in the left panel meets definition #1 when checked at 6 months and definition #2 when tested at 12 months after initiation of ACE-I. The right panel schematically demonstrates possible ways in which aldosterone breakthrough might or might not be manifested. This is derived from the meta-analysis of Bomback and colleagues and the work from North Carolina State University in an experimental model of RAAS activation. The ideal response to an ACE-I is that it falls and stays suppressed (~50% of human cases; red dashed line). In aldosterone breakthrough, RAAS suppression fails after a period of time (red and blue lines) and there is recrudescence of plasma (or urinary) aldosterone concentrations. This is observed in approximately 40-50% of human patients after 1 year of ACE-I therapy. There is evidence in experimental RAAS activation that aldosterone excretion in normal dogs is not demonstrably suppressed 6 hours post-treatment with ACE-I on days 1, 5 and 10 (represented in the black dashed line [a]), despite dramatic reduction in ACE activity.

Aldo = aldosterone; Mo = month.
Footnotes.


References.


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WHERE ARE WE WITH ALDOSTERONE ESCAPE (BREAK-THROUGH) IN 2011?


**Q & A - Clinical aspects of aldosterone and the «aldosterone-escape» concept: what is the role of aldosterone receptor blockade?**

Chair Jonathan ELLIOTT (JE) - *United Kingdom*

MA VetMB PhD CertSAC DipECVPT MRCVS

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**Panel:**

Allan D. STRUTHERS (AS)
BSc, MD, FRCP, FESC, FRSE, FMedSci - United Kingdom

Adriaan BOSWOOD (AB)
MA, VetMB, DVC, DipECVIM-CA (Cardiology), MRCVS - United Kingdom

Clarke E. ATKINS (CA)
DVM, DipACVIM (Internal medicine and Cardiology) - USA

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**Question (Chair) —** One of the conclusions from human clinical trials is that patients with not particularly elevated aldosterone levels may still benefit from mineralocorticoid receptor blockade. Why is this?

**Answer (AS) —** I suspect that there may be an important difference between plasma and tissue levels of both aldosterone and angiotensin II. There is also the problem with plasma levels being a crude measure of aldosterone and urinary concentrations may be more reliable. Thirdly, there is also the possibility that drugs may work through mechanisms other than aldosterone receptor blockade.

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**Question (David G. Warnock, MD, Director of the Division of Nephrology, Professor of Medicine and Physiology at the University of Alabama, Birmingham, USA) —** On the subject of aldosterone escape versus breakthrough, I prefer the latter term as it is much more descriptive and perhaps illuminating term as a description. Also, it might not simply be that tissue levels of aldosterone are different but that there are differences in tissue responsiveness. So, given problems associated with plasma (as opposed to urinary) measures of aldosterone and taking into account the notion of different tissue sensitivity, it may be a mistake to get too rigid about levels or changes when a patient could still benefit from treatment despite apparently suffering from aldosterone breakthrough?

**Answer (CA) —** A rigid definition is probably not a problem as in practice we do not use plasma aldosterone or Urinary Aldosterone to Creatinine ratio (UAC) to make judgements.
The philosophy behind mineralocorticoid blockade is good and I wonder whether we should routinely start patients on spironolactone when they go on to an ACE inhibitor.

**Answer (AS)** — Your comment about receptor responsiveness is good and explains why aldosterone levels might not matter. Variables include access to the receptor, competition from glucocorticoids, enzyme protection which is altered in states of oxidative stress. So there are many good reasons why aldosterone levels might not be that reliable. Furthermore, high aldosterone levels may be useful in patients with hyperkalaemia, and so aldosterone breakthrough is good in this instance.

**Question (Chair)** — From a pharmacological perspective, what dose of mineralocorticoid blocker should you use? Does the response change and how do you work out how much we need to block the RAAS? There’s a lot of variability in responsiveness of patients and we’re just starting to learn about that in veterinary medicine. What insights are there from human studies?

**Answer (AS)** — We tend to use the does from large clinical trials. But it is important to remember that genetic influences may determine the response to therapy.

**Question to AB (Delegate)** — These are wonderful studies. Did you measure plasma or urinary glucocorticoids in the studies because it may be important to understand which ligand of the mineralocorticoid receptor is important?

**Answer (AB)** — No, we didn’t measure glucocorticoids but still have the samples. It’s a great thought, maybe we should do it.

**Question to AB (Bertram Pitt)** — In humans a high cortisol and high aldosterone predicts a poor outcome. But age is a confounding factor: in humans aldosterone decreases with age and there is a decrease in the activity of the mineralocorticoid receptor protector enzyme (11 β-hydroxysteroid dehydrogenase), so cortisol plays an increasing role with age. So that’s one more reason to look at cortisol levels in your studies.

**Answer (AB)** — Again we did not measure cortisol but it is an excellent idea.

**Comment (Chair)** — Pharmacogenomics is a huge area and one in which we are way behind in veterinary medicine. Especially since dogs represent such a wide range of breeds with hugely different genetics.

**Question (Delegate)** — Historically aldosterone breakthrough was discovered with the chymase pathway, which leads to non-suppressed angiotensin II levels. How do we study this?
Answer (CA) — I’ve never studied it. Kallikrein is also one of the other alternative enzymes that can convert angiotensin I to II, so multiple things have been suggested. I was amazed going through the literature that we don’t know more about this mechanistically and we don’t have better definitions in human medicine for aldosterone breakthrough, so there’s clearly room to grow and learn more about this.

Answer (JE) — I understand that it would probably be more effective to look at the conversion of angiotensin I to II in patients rather than simply measuring ACE levels as a marker of the efficacy of your treatment.

Answer (AS) — We must also consider that non-ACE pathways may be influenced by treatment. People started using angiotensin receptor blockers and adding them to ACE inhibitors. The clinical trials in human patients trying that strategy have been a bit disappointing, except in maybe in some of the diabetic nephropathies. That made you wonder whether the chymase was contributing in most patients because you would expect adding an angiotensin II receptor blocker to an ACE inhibitor or using an angiotensin receptor blocker instead of an ACE inhibitor would produce huge benefits, but generally that’s not been the case.

Question (Delegate) — I worry that we don’t fully understand the physiology of aldosterone secretion. For instance, a diuretic will reduce potassium and so should lower aldosterone secretion, yet it activates the RAAS leading to aldosterone secretion. If we then block the RAAS with an ACE inhibitor, the only effect would be to suppress aldosterone but in dogs given frusemide, potassium drops. So, if aldosterone breakthrough occurs in animals, it can’t be due to changes in potassium and it can’t directly be explained it by the effect of ACE inhibitors, then it must clearly have another mechanism.

Answer (AS) — Yes, I agree, it is slightly paradoxical that furosemide puts the potassium down and yet it also puts the aldosterone up. One of the other important mechanisms for furosemide is the sodium in the macula densa. It’s not just potassium, it’s also sodium passing through the kidney which promotes renin release. That’s driven by renin, rather than by the potassium. But, I agree that it is complicated and not fully understood.

Question to AB (Delegate) — Have you looked at the effect of sampling site on UAC? We did a study in dogs at home and in the clinic and found a difference: UAC and catecholamine levels were higher in samples collected at the clinic in these dogs.

Answer (AB) — We didn’t look at that, but the majority of samples were collected at home.

Question to AS (Mark Oyama) — I was struck by what Dr Struthers said: if you think about angiotensin II receptor blockers, they don’t have the beneficial effects that an ACE inhibitor does. So what about the relative contribution of the renin-angiotensin-
Question (CA) — Why are dogs less prone to adverse effects of both types of drugs on blood pressure and potassium?

Answer (Bertram Pitt) — In human patients, most problems with potassium are due to concomitant renal disease, maybe it is not the same in dogs? If glomerular filtration rate is normal we don’t see potassium problems in our patients.

Comment (Faiez Zannad) — In human medicine the effect of both drugs on potassium is rather something beneficial actually, because we face much more risk of hypokalemia. Then about hyperkalemia, hyperkalemia alone without worsening renal function is very uncommon. Hyperkalemia is very often concomitant with worsening renal function. In human medicine, heart failure is very frequently on top of diabetes or hypertension with nephropathy which actually increases the risk of hyperkalemia. This is something that you probably do not see so often in veterinary medicine. This may explain the differences with human medicine.

Question (David G. Warnock) — What is the practice for dietary sodium intake?

Answer (AB) — In our study we did not recommend sodium restriction.
**Answer (CA)** — We recommend a standard diet with moderate sodium. We no longer prescribe low sodium diets to dogs with congestive heart failure; we have taken the ‘middle road’.

**Comment (Chair)** — There is the issue of what is a normal sodium intake as most commercial diets have a relatively high sodium level that would suppress aldosterone.
Faiez Zannad

MD, PhD
Professor of Therapeutics and Cardiology
Director, Clinical Investigation Centre, INSERM
Head, Heart Failure and Hypertension Unit
Department of Cardiology, CHU and University
Henri Poincaré,
Nancy, France.
Contact: f.zannad@chu-nancy.fr

Faiez Zannad is Professor of Therapeutics and Cardiology. He is at the Head of the Division of Heart Failure, Hypertension and Preventive Cardiology for the department of Cardiovascular Disease of the Academic Hospital (CHU) in Nancy and the Director of the Clinical Investigation Centre (Inserm-CHU) of Nancy since 1995. He entered the European Society of Cardiology (ESC) in 1996 and is currently the Chairman of the ESC Working group on Pharmacology and Drug Therapy as well as a Board member of the ESC Heart Failure Association. He is Past-President of the French Society of Hypertension.

As the Coordinator of French Cardiovascular Clinical Investigation Centres, he has participated in various famous large scale trials in human cardiology such as RALES, VALIANT, CIBIS, CAPRICORN, EPHESUS or EMPHASIS-HF. In these trials, he has been involved either as a member of the Steering Committees or in the Protocol Writing Groups.

He is Co-Editor-in-Chief for Fundamental and Clinical Pharmacology, the official journal of the European Pharmacology Societies Federation (EUPHAR). He chairs and organises annual international meetings on CardioVascular Clinical Trials (CVCT) and on Biomarkers in Heart Failure.
Clinical trials, where are we 10 years after RALES study?  
TARGETING THE ALDOSTERONE PATHWAY IN CARDIOVASCULAR DISEASE

Aldosterone is a key player in the pathogenesis of cardiovascular (CV) disease. Many details about the role of aldosterone in CV disease have, however, only recently been discovered and debate exists as to the relative importance of glucocorticoids and aldosterone in terms of mineralocorticoid receptor (MR) activation, which aldosterone modulator to use, which timing of treatment to aim for, and in which population to intervene. Accordingly, clinical trials have documented that blocking the MR-dependent effects of aldosterone can improve mortality and CV morbidity in patients with heart failure or myocardial infarction.

The greatest success with aldosterone blockade so far has clearly been in patients with heart failure (HF). Plasma aldosterone levels have been shown to be predictive of outcome in patients’ heart failure, irrespective of NYHA class, etiology and left ventricular ejection fraction. In the RALES trial severely symptomatic patients with systolic HF (NYHA III-IV), already treated with diuretics and an ACE inhibitor were randomized to receive either spironolactone 25-50 mg daily or placebo. Treatment with spironolactone was associated with a large reduction in both mortality and cardiovascular hospitalizations. Aldosterone blockade is a class I recommendation in North American and European HF guidelines as an important part of the treatment in patients with persistent class III-IV symptoms despite ACE-inhibitors and beta-blockers. Until recently, it was unclear if aldosterone antagonists would be effective in less advanced heart failure, and also if an effect similar to that seen in RALES would be evident in patients adequately treated with both ACE inhibitors and beta-blockers. In fact, a recent study in 226 HF patients in NYHA class II-III found that 9 months of eplerenone treatment on top of ACE inhibitors and beta-blockers did not improve symptoms nor did it reduce left ventricular dimensions. The levels of natriuretic peptides, were, however, decreased by eplerenone. In contrast, a clear effect on mortality and morbidity in this group was recently demonstrated in the much larger EMPHASIS trial. In the trial 2723 class II/III patients with LVEF ≤ 35 % and a recent hospitalization for HF or elevated levels of natriuretic peptides were randomized to eplerenone or placebo. The trial was stopped prematurely because eplerenone reduced the primary endpoint (CV death or HF hospitalizations) significantly by 34 %. Indeed, total mortality was also significantly reduced by eplerenone by 22 %. Although the precise position of aldosterone blockers in the treatment of less advanced heart failure is still debated, it is clear that a much larger role for these agents in class II patients than previously recommended is reasonable.

The mechanism behind the impressive effect of aldosterone blockade in chronic HF has been studied extensively. A recent meta-analysis of 9 randomized clinical trials showed
Clinical trials, where are we 10 years after RALES study? TARGETING THE ALDOSTERONE PATHWAY IN CARDIOVASCULAR DISEASE

a significant effect of drug treatment on left ventricular (LV) ejection fraction. Such an effect of aldosterone blockade has been documented also for patients who are already treated with angiotensin II blocking agents. In an MRI based study in 51 HF patients, treatment with spironolactone in addition to candesartan resulted in a significant improvement in LV end-diastolic diameter as well as in LVEF after 12 months. The effects of aldosterone blockade are not confined to LV volumes and systolic function. Indeed, an effect on measures of diastolic function of spironolactone in HF patients with preserved ejection fraction (HFPEF) has been documented. In experimental HF induced by chronic L-NAME infusion in rats, eplerenone further normalized diastolic function as measured by E/A ratio, independent of any effect on blood pressure. This would indicate that aldosterone blockade reverses early phases of HF and would emphasize the need for early intervention in the disease process. Similarly, administration of the active metabolite of spironolactone, canrenone, reduced myocardial norepinephrine content and increased the threshold for ventricular fibrillation in rats with experimental HF. Recently published experimental evidence supports that the myocardium becomes increasingly sensitized to mineralocorticoid excess in a setting of pressure overload leading rapidly to cardiac changes similar to those seen in HFPEF. In addition to the direct cardiac effect, aldosterone blockade might confer protection against development of the cardio-renal syndrome. Such an effect would be of tremendous clinical interest, since impairment of renal function in HF patients markedly increases mortality and morbidity.

In an experimental HF model in rats, the combination of spironolactone and an ACE-inhibitor was more effective than either alone, or than vehicle, to increase urine output and lower urinary protein excretion. Convincing data to prove a similar effect in humans are not yet available.

Following the publication of positive trials on aldosterone blockade in HF and rapid translation of trial results into clinical practice, concerns have been raised over the risks of adverse events, particularly hyperkalemia. The risk of hyperkalemia is highest in patients with renal dysfunction, high pre-treatment S-potassium, diabetes or prior use of antiarrythmics. However, if the inclusion criteria from RALES and EPHESUS are followed and appropriate monitoring is performed, the risk for serious hyperkalemia is very low if correct monitoring of S-potassium is performed. In the EPHESUS trial short term increase in S-potassium was not predictive of mortality and no deaths were adjudicated to hyperkalemia. Recent studies even indicate that the risk of serious hyperkalemia during treatment with spironolactone is low in oligouric hemodialysis patients. Taken together, the data suggest that the fear for clinically important hyperkalemia associated with aldosterone blockade may have been overemphasized. Probably even patients with tendency to hyperkalemia can be treated with aldosterone blockers if correct measures are taken.

Therapeutic tools and targets?

The CV consequences of MR activation may be overcome or reduced either by blockade at the receptor levels or by lowering the levels of
circulating agonists. As discussed above the vast majority of clinical evidence is based on the use of the MR blockers spironolactone and eplerenone. Very few direct studies comparing efficacy of spironolactone and eplerenone have been published, but a recent trial in hypertension due to primary hyperaldosteronism suggested that spironolactone might be more potent than eplerenone. This contrasts the findings of a previous smaller trial showing no difference. A recent study suggested that eplerenone was more effective than spironolactone in preventing hyperglycemia in HF patients, but doses used in the two arms were likely not comparable, in turn complicating the interpretation of the trial. There are no studies in HF comparing the effect on clinical outcomes of the two drugs. The main difference between the two drugs exists in the lack of sex steroid effect of eplerenone reducing or eliminating the risk of gynecomastia with this drug.

While treatment with MR receptor blockers is effective in reducing the effects of MR activation, it is also clear that treatment with for instance spironolactone or eplerenone increases levels of circulating aldosterone, and this could increase the degree of non-MR (non-genomic) mineralocorticoid effects. This feedback mechanism could be overcome by reducing aldosterone synthesis by inhibition of the CYP11B2 enzyme in the adrenal zona glomerulosa, which in turn would reduce mineralocorticoid MR activation. While aldosterone synthase inhibition represents an interesting potential target in the mineralocorticoid pathway, several questions remain to be answered. Firstly, there are potential risks which are not well described in large populations with CV disease, particularly the risks of hyponatremia and hyperkalemia. Secondly, it should be kept in mind that aldosterone synthase inhibition does not prevent glucocorticoids from activating the MR as discussed above, which could limit the utility of the drug in the absence of concomitant MR blockade. Further studies are required to address the potential of this group of compounds in the treatment of CV disease.

In clinical medicine the greatest development in the field has been initiating trials to test if expanding the indications for MR blockade will improve outcome in the entire spectrum of CV disease. Multiple hypertension trials have been completed or are enrolling, and trials in diabetic nephropathy are completed and underway. Given the multiple beneficial actions of MR blockers early in the processes leading to CV damage as discussed above, it appears that an outcome based trial targeting high risk individuals would be of considerable interest. As a consequence of the published evidence for using ACE-I and ARBs for this indication the trial should likely be a comparative study evaluating an aldosterone blocker to a blocker of the angiotensin II pathway, in a clinical setting of high CV risk, similar to the ONTARGET trial.

Evidence based treatment for heart failure with preserved ejection fraction (HFPEF) is essentially non-existing despite several trials attempting to demonstrate benefit from various pharmacological interventions. Most researchers consider HFPEF a vascular disease where longstanding increased peripheral resistance induces myocardial fibrosis and reduced ventricular and vascular compliance, in turn leading to non-systolic
heart failure. Given the well-described effects of MR blockade on both blood pressure and myocardial fibrosis, it could be hypothesized that MR blockers would be beneficial in HFPEF. This hypothesis is being tested in the large TOPCAT study randomizing patients with HF and LVEF > 45% to spironolactone or placebo. Another trial in HFPEF, the Aldo-DHF trial, is currently recruiting patients with class II-III HF, LVEF > 50% and echocardiographic evidence of abnormal diastolic filling. Patients are randomized to spironolactone 25 mg OD or placebo and the primary outcome measure is change in peak VO₂.

**Conclusion.**

Considerable amounts of knowledge about the importance of aldosterone and MR activation by aldosterone and glucocorticoids have emerged over the last decade. While intervention in the aldosterone pathway is already a crucially important step in the management of hypertension and HF, drugs to block the MR or interfere with aldosterone synthesis show promise in the treatment of other CV disease entities. Ongoing studies will address the optimal strategy to intervene successfully in the aldosterone pathway and in turn reduce the burden of CV disease.

**References.**

Bertram Pitt

MD
Professor of Medicine Emeritus,
University of Michigan, School of Medicine,
Ann Arbor, Michigan, USA.
Contact: bpitt@umich.edu

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).

Dr. Pitt 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, New England Journal of Medicine, and Lancet...). He was the principal investigator of RALES and EPHESUS, the co-principal investigator of EMPHASIS-HF and he is currently leading the large scale NHLBI TOPCAT trial investigating the clinical benefit of mineralocorticoid receptor blockade in patients with heart failure and a preserved left ventricular ejection fraction.
Clinical trials, where are we 10 years after RALES study?

TREATMENT OF PRESERVED CARDIAC FUNCTION HEART FAILURE WITH AN ALDOSTERONE ANTAGONIST: THE NHLBI TOPCAT TRIAL

The mortality and morbidity of patients with heart failure and a reduced left ventricular ejection fraction (HFREF) has decreased over the past decade due to the use of angiotensin converting enzyme inhibitors (ACE-Is) and or angiotensin receptor blocking agents (ARBs), beta adrenergic receptor blocking agents (BBs), mineralocorticoid receptor antagonists (MRAs), and implantation of devices including: implanted automatic cardiac defibrillators, cardiac resynchronization therapy (CRT), and most recently left ventricular assist devices (LVADs) for destination therapy. In contrast, the mortality and morbidity of patients with a preserved left ventricular ejection fraction (HFPEF) has changed relatively little.1 Unfortunately the incidence of HFPEF is increasing due to the aging of the population and the epidemic of visceral obesity in the western world.1 ACE-Is and/or ARBs and BBs while effective in patients with HFREF2,3 have had only equivocal results in patients with HFPEF. The effect of MRAs on mortality and morbidity in patients with HFPEF has however not been systematically evaluated. There is however reason to believe that a MRA both through its effects on the pathophysiology of HFPEF as well as on mechanisms associated with a number of important comorbid conditions may influence the outcome of these patients including: atrial fibrillation, diabetes mellitus, resistant or uncontrolled hypertension, obstructive sleep apnea, and chronic renal disease might be effective in altering the natural history of patients with HFPEF. The National Heart Lung and Blood Institute (NHLBI) has therefore initiated the TOPCAT trial4,5 the design and background of which will be briefly discussed below.

TOPCAT: study design.

3315 patients with a history of HFPEF will be randomized to the MRA spironolactone or placebo using a double blind protocol. To be eligible patients must be >/= 50 years of age with a LVEF </= 45% and either a history of hospitalization for HF within the previous year or a BNP >/= 100 pg/ml or NT-proBNP >/= 360 pg/ml within 60 days of randomization. Major exclusion criteria include uncontrolled hypertension, a prior history of serious hyperkalemia, an estimated glomerular filtration rate (e GFR) </= 30 ml/min/1.73 m², and or a serum potassium >/= 5.0 mmol/l. The primary endpoint is the composite of cardiovascular death, hospitalization for HF, or aborted cardiac arrest.

Randomized patients will be initiated on a starting dose of 15 mg/day of study drug and will be up titrated to 30 mg after one month if the serum potassium remains < 5.0 mmol/l. An additional up titration is allowed after 4 months at the investigators discretion if the serum potassium remains < 5.0 mmol/l and there are signs or symptoms of progressive HF. If at any time the serum potassium is >/= 6.0 mmol/l the study drug will be discontinued and if the serum
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Potassium is between 5.5 and 6.0 mmol/l the study drug will be down titrated to 15 mg /day. The study drug will also be discontinued in patients with an increase of serum creatinine to >/= 3.0 mg/dl.

Important ancillary studies include determination of ventricular function by echocardiography, determination of vascular stiffness by tonometry, and measurement of selected cardiac biomarkers. To date, more than > 3000 patients have been randomized into the study with a planned end of recruitment on January 31st 2012 and a subsequent follow up of one year so that the final results can be anticipated to be available in early 2013.

It should be emphasized that the dosing regimen in this trial is different from that with spironolactone in the RALES trial. In RALES patients were randomized to 25 mg of spironolactone or placebo and up titrated to 50 mg at one month whereas in TOPCAT patients will be started on 15 mg of study drug and up titrated to 30 mg at one month, and possibly to 45 mg at 4 months. An initial dose of spironolactone of 15 mg was chosen for this study since it was anticipated that many of the patients would be elderly and have concomitant diabetes mellitus and/or CKD, all of which could predispose to hyperkalemia.

Rationale to use Spironolactone in the treatment of HFPEF.

Prior data both from preclinical and clinical studies provide a strong rational for the use of a MRA in patients with HFPEF. Increasing evidence shows that myocardial fibrosis is critical in the transition from hypertensive or diabetic heart disease to HFPEF. Myocardial fibrosis is an early manifestation in patients with hypertension, visceral obesity, and or diabetes mellitus. Studies both in animals and man have shown that MRAs alone and or in conjunction with an ACE-I and/or ARB are effective in preventing myocardial fibrosis. There is a good correlation between serum levels of aldosterone and the degree of myocardial and vascular fibrosis as well as left ventricular mass.

The MRA spironolactone has been shown to improve the echocardiographic indices of diastolic function in patients with HFPEF. MRAs have also been shown to improve antioxidant reserves; reduce the formation of reactive oxygen species (ROS); reduce the formation of inflammatory cytokines and signaling through the NF kappa B and AP1 pathways; improve nitric oxide (NO) availability, endothelial function, and the number of circulating endothelial progenitor cells (EPCs); and reduce vascular stiffness and remodeling.

In addition MRAs have been shown to have a beneficial effect on the mechanisms associated with a number of important comorbid conditions in patients with HFPEF. For example, the MRA eplerenone has recently been shown to reduce the onset of atrial fibrillation /flutter in patients with NYHA class II HFREF in the EMPHASIS–HF trial, likely by preventing left atrial fibrosis and remodeling. Since atrial fibrillation is an important trigger for the transition from hypertensive or diabetic heart disease to HFPEF this finding may have important implications for the outcome of TOPCAT.

Aldosterone also appears to be of importance in patients with uncontrolled or resistant hypertension in that a MRA has been shown to significantly reduce blood pressure in these individuals at high risk for recurrent
HFPEF. Visceral obesity as mentioned above is also an increasingly important comorbid condition in elderly patients with HFPEF. The adipocyte releases substances such as Rac1 which stimulate the adrenal production of aldosterone with its potential adverse effects as outlined above. Serum aldosterone levels are also elevated in patients with obstructive sleep apnea, which is increasingly frequent in patients with HFPEF and visceral obesity.

Aldosterone has also been shown to increase albuminuria, podocyte damage, and mesangial cell fibrosis while MRAs decrease these pathological mechanisms\textsuperscript{16,17} which are important in CKD which is a frequent comorbid condition in patients with HFPEF. Aldosterone and/or MR activation are also important in diabetes mellitus and have been shown to cause pancreatic beta cell damage and insulin resistance.\textsuperscript{18}

Aldosterone and/or MR activation have also been shown to be important in atherosclerosis and MRAs have been shown to reduce the extent of experimental atherosclerosis in non-human primates\textsuperscript{19} as well as reducing the consequences of atherosclerosis such as stroke.

The fact that many patients with HFPEF are elderly and aldosterone levels are known to decrease with age\textsuperscript{20} has led to the speculation that a MRA might not be as effective in the very old as in younger patients despite their benefits as outlined above. However, there is also a decrease with age in the expression of the enzyme 11 Beta HSD2\textsuperscript{21} which converts cortisol, which can activate the MR, to cortisone, which can not, such that in the elderly cortisol may be an important activator of the MR. Recent data has also shown that MR expression in the vascular wall is increased with age.\textsuperscript{22} Thus, it is likely that despite a reduction in serum aldosterone levels, that activation of the MR is as or more important in elderly patients with HFPEF than in younger patients.

While as outlined above there is reason to believe that a MRA will be effective in reducing mortality and morbidity in patients with HFPEF the relatively high incidence of comorbid diabetes mellitus and or CKD in these patients increases the risk of hyperkalemia. Therefore, elderly patients with HFPEF need to be carefully screened for diabetes mellitus and or CKD, serially monitored for serum potassium and renal function, and the dose of the MRA adjusted accordingly. The development of new oral non-absorbable potassium binding polymers\textsuperscript{23} and new non-steroidal MRAs,\textsuperscript{24} which in preclinical studies appear to have a more favorable sodium/potassium ratio than either spironolactone or eplerenone, hold the promise that in the near future the risk of hyperkalemia associated with the use of a MRA might be reduced and that this strategy can be safely used in even higher risk patients with HFPEF and CKD.

**Conclusion.**

In conclusion, the TOPCAT\textsuperscript{4,5} trial holds the promise of altering the outcome of patients with HFPEF. Its success will however depend not only on the effectiveness of MRAs on the myocardium and vascular wall, the mechanisms associated with its important comorbid conditions as outlined above, the risk of hyperkalemia, but also on the conduct of the trial, which will be analyzed using the intent to treat principle, in maintaining compliance to the protocol and in preventing patients from dropping out.
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Michele Borgarelli

DVM, PhD, DipECVIM-CA (Cardiology)
Associate Professor
Kansas State University
College of Veterinary Medicine
1800 Denison Avenue
Manhattan, Kansas, USA.
contact: mborgarelli@gmail.com

Michele Borgarelli is Doctor in Veterinary Medicine, graduate of the Veterinary School of Torino in 1989. Since 1990 he has developed cardiology, abdominal ultrasound and internal medicine training in Italy, Europe and the USA. From 1995 to 2007 he was temporary Professor and then Assistant Professor (internal medicine and cardiology) at the Faculty of Veterinary Medicine University of Torino. Since 2009 he is Associate Professor of cardiology at Kansas State University. Diplomate of the European College Veterinary Internal Medicine (Cardiology) in 1999, Dr. Borgarelli earned in 2005 his PhD in clinical sciences with a thesis on “Mitral Valve Disease in dogs”.

Since 1991, he is member of the board of the European Society of Veterinary Cardiology (ESVC). From 2004 until 2007 he was President of Italian Society of Veterinary Cardiology. Since 2009, he is the President of the European College of Veterinary Internal Medicine.

Author of 60 scientific publications, Dr. Borgarelli is actually in clinical research programs on pathophysiology and therapy of heart failure, and chronic mitral valve disease in dogs.
Clinical trials, where are we 10 years after RALES study?

THE DELAY STUDY

(DELay of Appearance of symptoms of canine degenerative mitral valve disease treated with Spironolactone and Benazepril)

Chronic Degenerative Mitral Valve Disease (CDVD or DMVD) is the most common acquired cardiovascular disease in the dog representing 75% of all cardiovascular disease in these species. The disease is characterized by a long pre-clinical period. Asymptomatic dogs are a non homogeneous group, including patients with very mild disease and others that present a more advance disease and are more likely to develop clinical signs of HF, even though they have never developed HF. The heterogeneity of this group of dogs may be an important reason for the conflicting data concerning neuro-hormonal activation and efficacy of early treatment for DMVD presented in the veterinary literature for dogs with asymptomatic disease. Preliminary data from our laboratory showed that plasma aldosterone levels are significantly more elevated in asymptomatic affected dogs compared to a control group of healthy dogs. This observation suggests that aldosterone escape mechanism can be present in the early course of CDVD, and that aldosterone can play a role in the progression of the disease. It would also confirm data from dogs with experimentally induced CDVD suggesting that blocking renin-angiotensin system (RAAS) in dogs may necessitate multiple drugs such as an association of an ACE-I and spironolactone.

The recognition that asymptomatic dogs are an heterogeneous group of dogs underline the importance of developing some biomarkers able to identify the patients at higher risk for developing CHF, these patients could be the patients who may benefit from an early treatment. Proposed prognostic indicators for increased risk of death or progression of CDVD are represented by age, gender, intensity of heart murmur, degree of valve prolapse, severity of valve lesions, degree of mitral valve regurgitation and left atrial enlargement. Data from our group suggest that the left atrial enlargement represent the most independent predictor of progression or death for dogs with both symptomatic and asymptomatic disease. A recent study conducted on 72 asymptomatic dogs with MMDV showed that the N-terminal fragment of proBNP (NT-proBNP) is correlated with the severity of mitral regurgitation. In this study a cut off of 466 pmol/L had 80% sensitivity and 76% specificity with an area under the curve of 0.81 in predicting 12-month progression (cardiac death or HF). Recently troponin I (TnI) has been reported being associated with the severity of CDVD in dogs. In this study TnI has been suggested representing a biomarker of myocardial remodeling for this disease. Although this data appear very promising, further studies are needed.
Clinical trials, where are we 10 years after RALES study?

THE DELAY STUDY

to confirm the value of BNP and TnI in distinguishing, among asymptomatic dogs, those that will progress to HF.

These observations justify the investigation of benefit of spironolactone plus benazepril, through aldosterone blockade and ACE inhibition, in delaying the onset of HF caused by DMVD (positive effect on the clinical signs and mortality).

The DELAY study will include 240 dogs with advanced pre-clinical CDVD ISACHC class 1b or ACVIM consensus stage B2. The primary aims for this study are to evaluate the efficacy of spironolactone in combination with benazepril on delaying time of onset of overt heart failure and to evaluate if NT-proBNP and TnI are clinically relevant biomarkers to predict time of onset of heart failure. The study has started on November 2010 and enrollment will terminate on December 2012. End of the study is December 2015.

References.

**Q & A - Clinical trials, where are we 10 years after RALES study?**

Chair Jens HÄGGSTRÖM (JH) - *Sweden*
DVM, PhD, DipECVIM-CA (Cardiology)

**Panel:**
- Faiez ZANNAD (FZ)
  MD, PhD - *France*
- Bertram PITT (BP)
  MD, Professor of Medicine Emeritus - *USA*
- Michele BORGARELLI (MB)
  DVM, PhD, DipECVIM-CA (Cardiology) - *USA*

▶ **Question (Mark Oyama) —** Could I have the panel's hypothesis on the value of the primary antifibrotic effects of aldosterone blockade in diseases where there is little fibrosis? For instance in dogs with degenerative mitral valve disease where we think maybe there isn't a lot of fibrosis, how applicable are mineralocorticoid receptor blockers in these populations?

**Answer (FZ) —** There has been a focus on fibrosis because it is something that we can more easily measure. But it doesn’t tell the whole story, especially in other disease states. We have indications that the mechanism of action of aldosterone receptor blockers is really pleiotropic. They have many mechanisms of action: one of them is related to potassium, for example. We do have data on fewer hypokalemia when it comes to prevention of atrial fibrillation, but their effect on inflammation is also part of it... Just because a disease has little or no fibrosis, it doesn’t mean that the drugs won’t be effective.

**Answer (BP) —** Fibrosis is just one of many factors. Aldosterone, for instance, tremendously increases free radical production through its activation of mineralocorticoid receptors. So, measures of antioxidant reserve or free radical production will be very important in understanding drug therapy. If we give very potent antioxidants, we can turn off some of the effects of aldosterone. So I think that may be a clue for you.

▶ **Question to MB (Delegate) —** There would appear to be differences between naturally-occurring and experimentally-induced mitral valve regurgitation in dog? Why is this?
Is experimentally-induced mitral valve regurgitation a good model for the naturally-occurring disease, which is slow and progressive?

**Answer (MB)** — The use of milder models, with less surgical interference and where dogs can be followed for up to 3 years without treatment, means that we can mimic slower-progressing and less severe mitral valve insufficiency. But I don’t think that the mitral valve model can reflect the natural history of the disease, this is my personal opinion. We probably can look at the data we have in the naturally-occurring mitral valve regurgitation. Apparently there is a wide heterogeneity in the population of these patients.

**Question (Allan Struthers)** — On the subject of chymase, angiotensin I receptor blocker drugs have been used in humans to understand this pathway. Have they been used in dogs?

**Answer (MB)** — No, they haven’t been used in animals.

**Comment (Chair)** — I believe that Dell’Italia’s group* has some data on angiotensin-I blockers but the results are not encouraging. Although there do appear positive effects of blocking chymase in their dog model.

**Answer (MB)** — But, again this is in a model and not patients with naturally-occurring disease, which makes everything probably more complicated to interpret.

**Question (FZ)** — How common is atrial fibrillation in canine patients? Because, in humans, this is related to fibrosis?

**Answer (MB)** — Atrial fibrillation is common in large breed dogs (>20 kg), but less so in smaller breeds.

**Question to MB (Delegate)** — Would you consider looking at cases with renal failure in the DELAY study?

**Answer (MB)** — We would like to do this. But we have safety data about both drugs which do not show any problem. So we are just checking blood urea nitrogen and creatinine at the start of the study and only enrolling patients if these are normal.

**Question to MB (Delegate)** — How many asymptomatic dogs have severe DMVD?

**Answer (MB)** — Between 20% and 30%.

**Question to MB (FZ)** — How subjective is your measure of heart failure symptoms?

**Answer (MB)** — They are backed up by radiographic evidence of pulmonary oedema.

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* Dell’Italia’s team, Department of Medicine, Division of Cardiology, University of Alabama at Birmingham, Birmingham, Alabama, and Dillon’s team, Auburn University College of Veterinary Medicine, Auburn, Alabama.
Question (MO) — **What is the standard reduction in risk for acceptance of a clinical trial?**

*Answer (BP)* — Generally it is what is clinically meaningful. Our heart failure studies have been powered to detect a 15% reduction in risk.

*Answer (FZ)* — It is a compromise between sample size, methodology and what is clinically meaningful. Even a 5% risk reduction may be clinically meaningful.

Question (Delegate) — **Did you look in the big human studies at cardiac rhythm problems of patients? Is there a systematic evaluation of arrhythmias that might be prevented by mineralocorticoid receptor blockers?**

*Answer (BP)* — There is evidence of a reduction in sudden death, ventricular arrhythmias and ectopic activity, and calcium channel depolarisation. This suggests that these drugs are affecting atrial fribillation/flutter and ventricular arrhythmias.

Question (Adrian Boswood) — **On the subject of spironolactone versus eplerenone, with respect to glycaemic control, are patients that have been treated with spironolactone more likely to have type II diabetes mellitus?**

*Answer (FZ)* — The effect of these drugs is consistent across diabetic and normal patients, but there may be differences in subgroups of diabetic patients.

*Answer (BP)* — It may take years for differences in Haemoglobin A1c to play out clinically, but we do recommend using eplerenone in the diabetic sub-set of patients.

Question (JH) — **Could you comment on new non-steroidal mineralocorticoid blockers?**

*Answer (FZ)* — We are changing the molecules so that they have less steroidal-blocking effects and fewer side effects on the kidneys. This means that they are more cardiovascular-targeted and have less potassium and kidney issues. But we come back to the point that, even if aldosterone levels are normal, there is still a benefit from mineralocorticoid receptor blockade.

Question (Jonathan Elliott) — **Are there subtypes of mineralocorticoid receptors? For example, are the receptors in the kidney different?**

*Answer (FZ)* — Maybe it is a difference in expression rather than the type of receptor. That is the suggestion from really early data.
Adriaan Voors completed medical school in 1994 in Utrecht, the Netherlands. In 1997, he defended his PhD thesis entitled “Risk Factors, Endothelial Function and Clinical outcome after Coronary Bypass Surgery” (promotor: Professor W.H. van Gilst, University of Groningen, the Netherlands). He completed his training in internal medicine in Utrecht (Professor J.B.L. Hoekstra) and his training in cardiology in Nieuwegein, the Netherlands (Dr W. Jaarsma).

In July 2003, Dr. Voors started working as a Cardiologist, and became staff member of the department of cardiology of the University Medical Center Groningen. Currently, Dr. Voors is director of the Heart Failure Clinic and director of the Department of Echocardiography of the University Medical Center Groningen. In 2007, he became Established Clinical Investigator of the Netherlands Heart Foundation, Associate Professor of Cardiology, President of the Working group of Heart Failure and a board member of the Working group of Pharmacotherapy of the Dutch Society of Cardiology. In May 2010, he became Professor of Cardiology at the University Medical Center Groningen.

Professor Voors (co)authored more than 200 peer-reviewed papers and several books and chapters, mainly on heart failure, and he is deputy editor of the European Journal of Heart Failure and an editorial board member of the Journal of the American College of Cardiology, Netherlands Heart Journal and Cardiovascular Drugs and Therapy. He is principal investigator of 3 phase II heart failure trials, and executive/steering committee member of another 8 heart failure trials.
Biomarkers: what are the practical uses and what can be expected in the coming years?

HOW TO USE BIOMARKERS IN CARDIOLOGY?

Biomarkers have become increasingly important in the treatment of patients with cardiovascular disease. Due to major technological advances, it has become easier to find novel biomarkers that are increased in specific patients and their diseases. In heart failure, a large amount of biomarkers have become available and are can be used for several purposes.\(^1\)

1. Diagnosis.

Biomarkers might be of help in the diagnosis of several diseases, including heart failure. For example, in response to stretch or pressure, the heart releases natriuretic peptides.\(^2\) Therefore, greater concentrations of natriuretic peptides, such as BNP or NT-proBNP, indicate that the heart is “under pressure”. In patients that are admitted to the hospital with acute breathlessness, natriuretic peptides can be used to diagnose heart failure. In addition, several groups of markers might indicate the underlying disease. For example, heart failure is a syndrome of typical signs and symptoms, such as breathlessness and fatigue, caused by a functional or structural abnormality of the heart.\(^3\) However, there might be multiple causes for the impaired cardiac function. Biomarkers may help in finding the cause of heart failure. For example, an increase in inflammatory markers might indicate an inflammatory cause. Also, several markers of remodeling and collagen formation can be found in patients with hypertrophic heart disease. So, biomarkers might not only help in the diagnosis of heart failure, but its cause as well.

2. Hemodynamic status.

Several biomarkers might be used as indicators of hemodynamic status. For example, renal function (glomerular filtration rate) is strongly linked to the severity and prognosis of heart failure. A higher creatinine (lower GFR) indicates that there is either a decrease in cardiac output or an increase in central venous pressure.\(^4\) In addition, changes in natriuretic peptides might indicate changes in hemodynamic status.\(^2\) Several liver function tests might indicate whether there is an increased central venous pressure, or whether there is a decreased cardiac output.\(^5\) So, markers might help us to determine hemodynamic status of patients.

3. Prognosis.

Several biomarkers are elevated in patients with heart failure, and are strongly related to a poorer prognosis. For example, a higher level of serum sodium is an important predictor of a poorer prognosis. In addition, a poorer renal function is also clearly associated with increased mortality. Natriuretic peptides play an important role in the determination of the prognosis of patients with heart failure. Other markers, such as adrenomedullin\(^6\) and galactin-3\(^7\) might be even better in predicting prognosis in these patients. In the meantime, there are a large number of markers indicating prognosis, mainly due
to improving technologies to detect low abundant proteins. Therefore, a multimarker approach is currently advocated leading to better prognostic evaluation. With this approach, distinct biomarkers as a group might indicate those patients at the highest risk.


Biomarkers, and natriuretic peptides in particular, can be used to guide treatment. Patients with elevated levels of natriuretic peptides might be treated more aggressively in order to improve their prognosis. Several studies have been performed with mixed results. In particular, several studies have sought to investigate whether targeting natriuretic peptides would improve outcome. In other words, patients were randomized to standard treatment or to treatment related to natriuretic peptide levels. Some of these studies showed that BNP-guided therapy resulted in a better outcome, but others did not. The final goal of each marker predicting prognosis is to change clinical practice. In other words, it should be proven that when a biomarker is used to select or uptitrate therapy, that it improves outcome as well.


Biomarkers might also be used to select patients for a specific treatment. For example, in patients with high renin levels, treatment of ACE-inhibitors is highly effective to reduce blood pressure, while in patients with low levels of renin, diuretics are much more effective.

References.

Mark A. Oyama

DVM, DipACVIM (Cardiology)
Professor of Cardiology
Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, USA.
Contact: maoyama@vet.upenn.edu

Mark Oyama is a Professor in the Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania. His main clinical and research interests concern 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 a past President of the American College of Veterinary Internal Medicine, Specialty of Cardiology and 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 his wife, a Golden Retriever, and two very mischievous cats.
Biomarkers: what are the practical uses and what can be expected in the coming years?

POTENTIAL MARKERS OF CARDIAC REMODELING AND FUNCTION

Biomarker testing represents an attractive diagnostic and monitoring technique in dogs with mitral valve disease (MVD) and dilated cardiomyopathy (DCM). Compared to echocardiography and radiography, marker testing is potentially more cost effective and accessible to general practitioners. Pertinent to the topic of this symposium, markers of cardiac stress, remodeling, and function might offer a means to stage disease severity, monitor response to therapy, and provide information regarding risk of morbidity and mortality. Studies involving NT-proBNP have already demonstrated utility in stratification of risk for morbidity and mortality (Moonarmart et al., Serres et al., Chetboul et al.). These studies are particularly important as they move beyond the simple description of elevated markers in dogs and cats with heart disease. They offer information that is otherwise unavailable from conventional diagnostics.

Currently investigated biomarkers in canine medicine.

An incomplete list of candidate markers in dogs includes markers of necrosis (troponin and high sensitivity troponin [Ljungvall et al.]), remodeling and fibrosis (N-terminal procollagen type III [Hezzell et al.]; matrix metalloproteinases [Ljungvall et al.]); serotonin [Arndt et al.]), calcium handing (sodium-calcium exchanger [Nam et al.]; phospholamban [Lee et al.]), neurohormonal activation (copeptin [Oyama et al.]; urocortin [Veloso et al.]), and inflammation (C-reactive protein [Rush et al.; Ljungvall et al.]). To date, most veterinary studies have been limited to description of marker concentrations in populations of affected animals or the use of markers to diagnose presence or absence of heart disease. As previously mentioned, the next stage of investigation, wherein markers are found to offer risk stratification and treatment guidance, has just begun. In this respect, veterinary medicine is not so far behind our physician colleagues (see below).

Biomarkers for guided therapy.

The validation of markers is relatively laborious, insofar as studies evaluating their prognostic value requires longitudinal follow-up of relatively large cohorts of animals. In addition, for such markers to have the greatest clinical impact, validation of markers requires simultaneous advances in therapy such that once identified, the natural history of at-risk individuals can be altered by therapy or intervention. For instance, in dogs with MVD and persistently elevated NT-proBNP, would additional diuretics, beta-blockade, AT receptor blockade (or any other drug for that matter) improve outcome? Would this improvement be reflected in lower NT-proBNP concentration so that clinicians would know they have mitigated risk? In humans, meta-analysis indicates that marker-guided therapy reduced all-cause mortality by 30%
Biomarkers: what are the practical uses and what can be expected in the coming years?

POTENTIAL MARKERS OF CARDIAC REMODELING AND FUNCTION

(Felker et al.). The benefits appear greatest in patients that are younger and with systolic dysfunction vs. those >75yrs of age or with preserved ejection fraction.

The multimarker approach.

In human medicine, much attention has been given to the potential utility of combinations of markers (the so-called “multimarker” technique). Theoretically, multiple markers, each specific to various pathologic features of heart disease (i.e., fibrosis, ischemia, wall stress, extracellular matrix remodeling, etc) would offer more information than any single marker alone. Multimarker use in veterinary medicine already is widely used. Take for example a hepatic panel involving ALT, AST, ALP, GGT, and bile acids: each one of these markers provides slightly different information, and the combination of results typically is more useful than any one marker alone. Note that the hepatic markers are not necessarily definitive for any specific disease, but rather, the results increase the level of suspicion that various disease states (i.e., inflammation, bile stasis, etc) exist. Markers also provide the impetus to pursue additional and more definitive diagnostics (i.e., ultrasound, biopsy, etc). Thus, it is not particularly surprising that combinations of cardiac markers add prognostic information to existing risk factors. For example, in patients undergoing marker testing for NT-proBNP, C-reactive protein, cystatin-C, and cardiac troponin-I, the risk of cardiovascular death increased by 3-, 7-, and 16-fold depending on whether 2, 3, or all 4 markers were elevated (Zethelius et al.). The individual markers need not be particularly complicated or original. In humans, in addition to NT-proBNP concentration, consideration of serum glucose and estimated glomerular filtration rate yielded better prediction of mortality that any of the three alone (Damman et al.).

Challenges in canine medicine.

One challenge facing biomarker science involves the unique nature of canine MVD as compared to ischemic Mitral Regurgitation (MR), DCM, or myocardial infarction. Interestingly, experimental canine MVD is devoid of the extensive degree of remodeling and fibrosis that is seen in ischemic disease (Dell’italia et al.). Studies in canine MR demonstrate a net loss of collagen structure, and serum pro-collagen III concentrations are decreased in dogs with advanced MVD and eccentric hypertrophy (Zheng et al., Hezzel et al.). In dogs with naturally-occurring MVD, myocardial fibrosis is related to the presence and extent of arterial narrowing, and both parameters appear related to survival (Falk et al.). Thus, it is possible that myocardial fibrosis in MVD is actually related to small vessel hypertrophy and ischemia rather than an intrinsic characteristic of volume overload hypertrophy itself. Additional studies are required to determine if markers of fibrosis (and ischemia) will be useful in dogs with MVD.

Another challenge facing the identification of markers involves the apparently late activation of neurohormonal axes such as the circulating renin-angiotensin-aldosterone system in dogs with MVD (Fujii et al., Häggström et al.); however another study (Borgarelli et al.) reported elevated plasma aldosterone in dogs with advanced asymptomatic MVD. Thus, the exact timeframe and sequence of compensatory activity is poorly understood, and biomarker studies, in addition to their diagnostic and prognostic potential, can add to our knowledge about the pathophysiology of disease.
**New opportunities.**

To attempt to identify promising candidate markers for further study, the author has recently evaluated a panel of 8 novel markers including markers of platelet adhesion (E-selectin, ICAM-1), neurohormonal activation (copeptin, chromogranin A), and hypertrophy, fibrosis, and growth (galectin-3, ST2, osteopontin, endoglin) in approximately 200 dogs with naturally occurring disease. Pilot results from these studies will be discussed during the presentation. Based on these results, the author believes that certain classes of markers will be more useful than others, perhaps due to the unique characteristics of MVD and volume overload.

**References.**

Borgarelli et al. ACVIM Forum 2011, Denver CO

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Q & A - Biomarkers: what are the practical uses and what can be expected in the coming years?

Chair Jens HÄGGSTRÖM (JH) - Sweden
DVM, PhD, DipECVIM-CA (Cardiology)

Panel:

Adriaan A. VOORS (AV)
MD, PhD - Netherlands

Mark A. OYAMA (MO)
DVM, DipACVIM (Cardiology) - USA

Question (Delegate) — With regards to troponin levels, what are detection limits? We have seen in our studies that there is a subset of dogs at least, which has circulation defects and that are actually resembling the model you were referring to, where there is an increase of troponin levels as disease progresses.

Answer (MO) — Yes, we are surprised by the troponin results too. We don’t have a good explanation about why our cohort of dogs didn’t have a significant increase of troponin levels. We used a test whose lower reference range was around 0.01.

Answer (AV) — Troponin was first thought of as a marker of damage or injury but is now known to be present in all cases regardless of cause heart failure. It’s probably not really only ischemia that it reflects, but it might also reflect other things. We notice this as the assays get more and more sensitive, so at the moment we are confused about troponin and we use it as a prognostic marker, but we come up to the same discussion. For really detecting whether there is ischemia or not, it might not be the best marker.

Comment (Chair) — People who work on troponin in big cohorts and who look at really low troponin concentrations, changes in low concentrations and prognostication of an increased risk of developing myocardial infarction, they also don’t know what to do with troponin information. So it’s probably something that’s going to come more in the future I think.

Question to MO (Delegate) — In your data, markers of sympathetic nervous system activation, namely chromogranin A, was not of use whereas in humans it is of great
Q & A - Biomarkers: what are the practical uses and what can be expected in the coming years?

use. Are there differences between dogs and humans in relation to sympathetic nervous system activation? Is it in dogs that there is removal of parasympathetic tone rather than an increase in sympathetic activity?

**Answer (MO)** — This raised several questions. First does the biomarker test kit detect canine chromogranin A, or is it picking up something else? From our studies it would appear to be specific for chromogranin A. Second, it is possible that there is not much sympathetic nervous system activation in mild disease or, thirdly, that there is a disconnect between disease and amount of chromogranin A that dogs produce compared to people with mitral valve disease. This speaks maybe to the pathophysiology. I don’t dispute that advanced heart failure is typified by high sympathetic nervous system tone. So I don’t have a real good answer for why this cohort didn’t particularly show this marker. But in thinking more and more about canine mitral valve disease, things are beginning not to surprise me, they act differently than everything else that we know.

**Comment (Chair)** — I would like to stress that there are difficulties in assessing sympathetic nervous system activation, measuring epinephrine, norepinephrine and catecholamines in dogs, as they are inherently unstable in plasma samples.

**Answer (AV)** — Urine is a better medium, they are more stable in that environment.

**Comment (Delegate)** — We have had good results performing ELISA analysis of epinephrine and norepinephrine in canine urine. There are also groups in the Netherlands that read about us who have been using this. So I think this is quite promising.

**Question to MO (Delegate)** — Was there any difference between dogs with Dilated Cardiomyopathy (DCM) and Degenerative Mitral Valve Disease (DMVD)?

**Answer (MO)** — There was less than 10 dogs with dilated cardiomyopathy in our study, so it was not possible to answer that. But I think it might be different, particularly with activation of sympathetic nervous system, maybe in DCM more than in DMVD.

**Question to AV (Adrian Boswood)** — With regards to biomarker guided therapy, it is my understanding that meta-analysis shows improvements in large numbers of patients?

**Answer (AV)** — This requires caution because close inspection reveals the peculiar finding that the smaller the study, the greater effect, and vice versa.

**Comment to AV (Adrian Boswood)** — When using the area under the curve to assess the value of markers, this doesn’t appear to take into account the time to an event. So depending on the period over which you’re looking at the prognostic power of the index, you may be losing information then.

**Answer (AV)** — The data that I presented were based on fixed time studies. The reality is that prognostic markers must be better.
Question to MO (Rebecca Stepien) — If some combination of markers has prognostic value and you know that a patient - whether it is a human or dog - is going to go into heart failure before the next visit what do you do? Do you monitor more frequently or what?

Answer (MO) — To answer that we need more research. We need to stratify, randomise and treat a number of dogs and study what happens. Or, do we simply ask owners to be more vigilant?

Answer (AV) — We stimulate each other to test the hypothesis.

Question to MO (Delegate) — Did you check your panel of markers against disease severity?

Answer (MO) — There was no discernible differences across disease severity groups.
Rebecca L. Stepien

DVM, MS, DipACVIM (Cardiology)
Clinical Professor of Cardiology
Department of Medical Sciences
University of Wisconsin
School of Veterinary Medicine
Madison, Wisconsin, USA.
Contact: stepienr@vetmed.wisc.edu

Dr. Rebecca L. Stepien graduated from the University of Wisconsin School of Veterinary Medicine and completed a cardiology residency at the Ohio State University. She subsequently taught at the Royal Veterinary College in London and the Virginia-Maryland Regional College of Veterinary Medicine. She is currently a Clinical Professor at the University of Wisconsin School of Veterinary Medicine, where she has taught and seen referral patients since 1994. Dr. Stepien holds a Master’s degree in Clinical Sciences, is board-certified in the specialty of Cardiology in the American College of Veterinary Internal Medicine and is a past president of the ACVIM Specialty of Cardiology.

Her academic and clinical interests include diagnosis and therapy of systemic hypertension, mitral valve disease in whippets, therapy of heart failure and veterinary ethics. Dr. Stepien is a frequent speaker at international veterinary conferences and in 2008, was awarded the British Small Animal Veterinary Medical Association Bourgelat Award for outstanding contribution to the field of small animal practice.
Cardio-Renal Syndrome, what is behind it?

A ROCK AND A HARD PLACE: CARDIORENAAL SYNDROME IN CLINICAL CANINE VETERINARY PATIENTS

Myxomatous or degenerative valve disease is the most common type of heart disease in dogs and typically occurs in middle-aged to older dogs. Dogs with renal dysfunction are also typically in the adult to geriatric age range (Jacob et al. 2003) and the risk of renal dysfunction may be increased by the presence of medical therapy for congestive heart failure (CHF) (Butler et al. 2004; Sayer et al. 2009).

Definition and pathophysiology.

Cardiorenal syndrome has been variously defined. In clinical medicine, it may be viewed as “a state in which therapy to relieve heart failure symptoms is limited by worsening renal function” (NHLBI Working Group, http://www.nhlbi.nih.gov/meetings/workshops/cardiorenal-hf-hd-htm), but this arguably is a clinical view reflecting a cardiologist’s concern; a nephrologist may be more likely to define CRS as “…a normal kidney that is dysfunctional because of a diseased heart…” (Ronco et al. 2008). An expansive, multisystem view is required in order to understand the complex bidirectional interactions of these two critical body systems, in which “…each dysfunctional organ has the ability to initiate and perpetuate disease in the other organ through common hemodynamic, neuro-hormonal, and immunological/biochemical feedback pathways” (Bock and Gottlieb 2010).

The “low flow” theory of how cardiac dysfunction leads to renal dysfunction involves decreased cardiac output leading to decreased renal perfusion, which results in renin release from the juxtaglomerular cells and pressure-sensing baroreceptors. Renin-angiotensin-aldosterone system (RAAS) activation causes retention of sodium, volume retention, afferent arteriolar constriction that decreases GFR, and release of profibrotic neurohormones that lead to ventricular remodeling (Bock and Gottlieb 2010). However, studies of therapies to improve cardiac index and decrease pulmonary capillary wedge pressure (i.e. effective treatments of cardiac dysfunction) have shown that these therapies do not predict improvement in renal function (Weinfeld et al. 1999; Mullens et al. 2008; Mullens et al. 2009) and therefore, are inadequate to address this complex issue. A more specific understanding of the physiologic parameters involved in maintaining constant blood volume and organ perfusion promotes better understanding of their relationships, and perhaps better targets for therapy. Although understanding of the physiologic parameters involved in CRS are still incomplete, there is general recognition that elevation of intra-abdominal and central venous pressure, increased sympathetic nervous system activity, RAAS dysfunction, oxidative and endothelial dysfunction, renal effects of arginine vasopressin and adenosine and in some patients, decreases in erythropoietin contribute variably to CRS in a given patient (Bock and Gottlieb 2010).

Cardiorenal syndrome may be divided into 5 types (Ronco et al. 2008).
Cardiorenal syndrome and its significance in canine medicine.

Type 2 CRS appears to be of significant clinical concern in cardiac patients. Approximately 30% of >105,000 people admitted for acute decompensated heart failure had a history of renal insufficiency and approximately 21% of them were azotemic (Adams et al. 2005). Decreased creatinine clearance is common in Congestive Heart Failure (CHF) patients, and affected almost 40% of those human patients with NYHA Class IV heart failure in one study (Adams et al. 2005). When worsening renal function (WRF) is defined as an increase in serum creatinine of >0.3 mg/dl compared to baseline, 27% of 1004 human patients admitted for heart failure developed WRF in the hospital, and in these patients, WRF was associated with worse outcomes even when the increase did not cause creatinine to exceed normal reference range (Forman et al. 2004). The sensitivity and specificity of an increase in creatinine ≥ 0.3 mg/dl with a final creatinine of ≥ 1.5 mg/dl for prediction of in-hospital mortality was 73% and 72% respectively, and this combination was seen in 29% of acutely decompensated human cardiac patients (Gottlieb et al. 2002). Higher creatinine concentrations at admission, as well as higher doses of loop diuretics and use of certain vasodilators increases the risk of worsening renal function in hospitalized human heart failure patients (Butler et al. 2004). Over a range of increases in creatinine (≥ 0.1 to ≥ 0.5 mg/dl), renal deterioration tended to occur within the first 3 days of hospitalization, regardless of total length of stay (Gottlieb et al. 2002).

The prevalence of azotemia and decreased renal function in canine CHF patients is similarly high. In a report in 2010, 24.1% of 223 dogs with heart disease were azotemic, although the prevalence of CHF in this cohort of heart patients was not specified (Ohad et al. 2010). In an earlier study, Nicolle and colleagues reported that 50% of small dogs (<13 kg) with degenerative valve disease were azotemic either by Blood Urea Nitrogen (BUN)
or Creat or both. This study included dogs in all stages of heart failure (NYHA grades I-IV) and some were treated at the time of the study. As their NYHA class increased, a greater percentage of patients were azotemic and were also more likely to have already been treated with some combination of angiotensin-converting enzyme inhibitors, furosemide, spironolactone and digoxin. Older dogs were also more likely to be azotemic. The severity of CHF was also linked to renal function; Class III-IV dogs had 45% decrease in GFR compared with Class I-II dogs, and 7/9 grade III-IV dogs had abnormally decreased GFR (Nicolle et al. 2007).

In human (Butler et al. 2004) and canine patients, the presence and severity of RAAS activation and azotemia is affected by therapy, especially furosemide and vasodilators. In normal beagles, the combination of a low sodium diet and “heart failure” doses of furosemide (2 mg/kg PO q 12 hrs) resulted in measurably higher renal-angiotensin-aldosterone system (RAAS) activation than a low sodium diet alone (Lovern et al. 2001). In 12 King Charles Spaniels with NYHA Class III CHF treated with enalapril for 3 weeks, no change was seen in angiotensin II (ATII) concentration and aldosterone concentration decreased, but these findings (suggesting limitation of RAAS activation) disappeared with the addition of furosemide to their therapy (Häggström et al. 1996). After 4 months of enalapril plus furosemide therapy, both aldosterone and ATII concentrations were significantly increased compared to baseline. The plasma BUN of dogs receiving enalapril monotherapy for 3 weeks was unchanged from baseline, but mean BUN of these dogs after furosemide had been added reached azotemic concentrations at the 6 month evaluation. Both hydralazine (Häggström et al. 1996) and amlodipine (Atkins et al. 2007) administration lead to measurable RAAS activation.

Anecdotal evidence supports the simultaneous occurrence of cardiac and renal dysfunction in clinical canine patients with valvular disease. Patients may be presented with clinical signs of CHF and evidence of pre-existing renal dysfunction diagnosed by detection of azotemia at the time of presentation. More commonly, however, renal dysfunction develops over time in cardiac patients, often increasing subtly but incrementally over the course of cardiac disease progression, then increasing more obviously when cardiac therapy involving furosemide and some vasodilators begins. In some cases, inadequate therapy of venous congestion may contribute to renal dysfunction, resulting in “congestive kidney failure” (Mullens et al. 2009).

Human and veterinary literature supports the use of ACEI and aldosterone receptor antagonists such as spironolactone to limit the RAAS activation that has been documented with CHF therapy (CONSENSUS 1987; RALES 1996; Ettinger et al. 1998; Ovaert et al. 2009; Bernay et al. 2010), and use of these modalities has prolonged survival times in affected patients. The topic of cardiorenal syndrome is becoming an increasingly important concern in clinical veterinary medicine. Avoidance of WRF in dogs before and during CHF therapy is crucial to maintain quality of life as survival in dogs with this common disease increases.
Cardio-Renal Syndrome, what is behind it?
A ROCK AND A HARD PLACE: CARDIORENAL SYNDROME IN CLINICAL CANINE VETERINARY PATIENTS


Claudio Ronco

MD

Director, Department of Nephrology,
Dialysis and Transplantation
International Renal Research Institute
St Bortolo Hospital, Vicenza, Italy.
Contact: cronco@goldnet.it

Professor Claudio Ronco graduated in medicine at the University of Padua, Italy, in 1976. He specialized in nephrology at the University of Padua in 1979, and in paediatric nephrology at the University of Naples in 1989. 1977-1998, he was Assistant and Associate professor at the Division of Nephrology in Vicenza. During the 1999-2001 period, Director of the Renal Laboratory at the Renal Research Institute and Professor of Medicine at the Albert Einstein College of Medicine and Beth Israel Medical Centre of New York. Since 2002, Director of Department of Nephrology at St. Bortolo Hospital, Vicenza, Italy.

Claudio Ronco has co-authored 994 papers, 85 book chapters and 62 books, and he has delivered more than 650 lectures at international meetings and universities. He is a council member of several scientific societies and Editor Emeritus of the International Journal of Artificial Organs. He is also Editor-in-Chief of Blood Purification and Contributions to Nephrology.

Professor Ronco has received numerous honours and awards including, in 2004, the Lifetime Achievement Award and honorary membership in the Spanish Society of Nephrology, the National Kidney Foundation International Medal of Excellence and in 2009, the ISN Bywaters Award for Acute Renal Failure.
Cardio-Renal Syndrome, what is behind it?

CARDIO-RENA L SYNDROMES: LESSONS FROM HUMAN PATHOPHYSIOLOGY

Cardiorenal Syndromes in Humans.

Patients admitted to hospital may present various degrees of heart and kidney dysfunction. Primary disorders of one of these two organs often result in secondary dysfunction or injury to the other. Such pathophysiological interactions represent the pathophysiological basis for a clinical entity often referred to as the Cardio-Renal Syndrome (CRS). Although generally defined as a condition characterized by the initiation and/or progression of renal insufficiency secondary to heart failure, the term CRS is also used to describe the negative effects of reduced renal function on the heart (renocardiac syndrome). The absence of a clear definition and the complexity of heart and kidney interactions contributed in the past to lack of clarity with regard to diagnosis and management. The most recent definition includes a variety of conditions, either acute or chronic, where the primary failing organ can be either the heart or the kidney. “Cardio-Renal Syndromes” (CRS) are thus disorders of the heart and kidneys whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction of the other. The current definition has been expanded into five subtypes whose etymology reflects the primary and secondary pathology, the time-frame and simultaneous cardiac and renal co-dysfunction secondary to systemic disease (table 1). Such advances in the definition and classification of CRS allow to characterize the complex organ crosstalk and have proposed specific prevention strategies and therapeutic interventions to attenuate end organ injury.

A major problem with previous terminology was that it did not allow to identify the pathophysiological interactions occurring in the different types of combined heart/kidney disorder. A large number of direct and indirect effects of each organ dysfunction can initiate and perpetuate the combined disorder of the two organs through a complex combination of neuro-humoral feedback mechanisms. For this reason a subdivision into different subtypes seems to provide a more concise and logically correct approach to this condition. This classification has been the result of a consensus conference held in Venice in 2008 under the auspices of the Acute Dialysis Quality Initiative (ADQI). Several experts from the fields of internal medicine, cardiology, cardiac surgery, nephrology and intensive care, debated the topic in a well established process for consensus. The advantage of this result is, not only the definition classification system that represents a great outcome itself, but also the initiation of a multidisciplinary collaboration towards new diagnostic, preventive and therapeutic strategies for patients suffering from combined disorders of the heart and the kidney. Such multidisciplinary approach requires a structured education of young physicians that should evolve in their careers with an open mind and an attitude more patient-oriented.
Cardio-Renal Syndrome, what is behind it?

CARDIO-RENAL SYNDROMES: LESSONS FROM HUMAN PATHOPHYSIOLOGY

rather than organ oriented. The birth of this new journal may represent the basis for this innovative trend and for a new era in the management of cardio-renal patients.

Cardio-renal Syndrome type 2: a condition close to veterinary pathophysiology.

Type II CRS or chronic Cardio-Renal Syndrome (CCRS) is characterized by chronic abnormalities in cardiac function causing progressive chronic kidney disease (CKD). Worsening renal function (WRF) in the context of heart failure (HF) is associated with significantly increased adverse outcomes and prolonged hospitalizations. The prevalence of renal dysfunction in chronic heart failure (CHF) has been reported to be approximately 25% in humans. Even limited decreases in estimated GFR of > 9 ml/min appears to confer a significantly increased mortality risk. Some researchers have considered WRF a marker of severity of generalized vascular disease. Independent predictors of WRF include: old age, hypertension, diabetes mellitus and acute coronary syndromes.

Chronic HF is characterized by a relatively stable long-term situation of probably reduced renal perfusion, often predisposed by both micro- and macrovascular disease in the context of the same vascular risk factors associated with cardiovascular disease. No evidence of association between LVEF and estimated GFR can be consistently demonstrated. Neuro-hormonal abnormalities are present with excessive production of vaso-constrictive mediators (epinephrine, angiotensin, endothelin) and altered sensitivity and/or release of endogenous vasodilatory factors (natriuretic peptides, nitric oxide).

Recently, there has been increasing interest in the pathogenetic role of erythropoietin (EPO) deficiency and decrease in Vitamin D receptors activation. Regardless of the cause, WRF in the context of heart failure is associated with increased risk for adverse outcomes. The proportion of individuals with WRF or CKD receiving appropriate risk factor modification and/or interventional strategies is lower than the general population. Potential reasons for this therapeutic failure include concerns about worsening of residual renal function, and/or therapy-related toxic effects due to low clearance rates. However, several studies have shown that when appropriately titrated and monitored, cardiovascular medications used in the general population can be safely administered to those with renal impairment and with similar benefits.

Newer approaches to the treatment of cardiac failure such as cardiac resynchronization therapy (CRT) have not yet been studied in terms of their renal functional effects, although preserved renal function after CRT may predict a more favorable outcome. Vasopressin V2-receptor blockers have been reported to decrease body weight and edema in patients with CHF, but their effects in patients with CRS have not been systematically studied and a recent large randomized controlled trial showed no evidence of a survival benefit with these agents.

It has been recognized the difficulty to distinguish in advanced stages whether patients belong to type II or type IV CRS. This however should not be matter of concern since the classification system describes clearly that patients may move among different CRS subtypes along the natural history of the syndrome.
References.


Frédéric Jaisser

MD, PhD
Research Director - INSERM
(French National Institute of Health and Medical Research).
Paris, France.
Contact: fréderic.jaisser@inserm.fr

Frédéric Jaisser is Research Director at the Unit 872 of INSERM (Institut National de la Santé et de la Recherche Médicale), the French public research institution entirely dedicated to human health. He is Professor at the Faculty of Medicine of Reims where he coordinates different courses such as « Animal Models and Pathophysiological Mechanisms ». Since 2010 he is the Scientific coordinator of the Pathophysiology committee at The National Research Agency.

He is MD, specialist in Nephrology and has a University degree in Biological and Medical Engineering. He joined INSERM in 1996. His fields of expertise are mainly renal and cardiovascular pathophysiology, development of transgenic animal models for pathophysiologic studies or human disease models. He is also the coordinator of several multicentric projects.

He is an Editorial Board Member of Endocrinology and an expert for several other peer-review journals such as Circulation or Hypertension. He is currently the President of ESAC-France (European Section of the Aldosterone Council).
Cardio-Renal Syndrome, what is behind it?

MINERALOCORTICOID RECEPTOR ANTAGONISTS: NEW THERAPEUTIC OPPORTUNITIES IN CHRONIC KIDNEY DISEASES

Slowing the progression of chronic kidney diseases needs new efficient treatments. Blocking the renin-angiotensin-aldosterone system, especially using angiotensin receptor blockers, has proven to be effective to decrease proteinuria in several renal diseases. Treatment targeting aldosterone and its receptor, the mineralocorticoid receptor, is another option that should be considered. There is a compelling indication for the use of mineralocorticoid receptor blockers (MRBs) for patients with heart disease; prospective randomized controlled trials have demonstrated reduction in mortality in patients with severe heart failure, for those who develop heart failure following acute myocardial infarction and those with mild heart failure. To date, patients with CKD have not been included in large-scale prospective outcome studies with MRBs, primarily because of concerns about hyperkalemia.

The aim of this short review is to consider the implications of aldosterone, the MR and MRBs in the progression and treatment of chronic kidney disease.

New concepts on the pathophysiological role of aldosterone.

Aldosterone controls sodium reabsorption and potassium secretion in the distal nephron. Aldosterone thus plays a major role in volume and blood pressure homeostasis. The hormone acts in the distal nephron after binding to the MR, a ligand-dependant transcription factor which binds to specific hormone response elements. Molecular targets whose expression is modulated by aldosterone in the distal nephron are numerous. MR is expressed in the distal nephron (convoluted distal tubule and collecting duct), distal colon and sweat glands, all sites previously known as classical targets of aldosterone and the regulation of renal sodium reabsorption. In the kidney, MR is also expressed in non-epithelial kidney cells such as mesangial cells, podocytes and renal fibroblasts. Therefore, MR is expressed in tissues and cell types where vectorial sodium transport does not occur, indicating novel and yet unknown roles of aldosterone and MR activation in these targets that do not serve whole body sodium homeostasis. MR expression is not fixed, but can be modulated in various pathophysiological contexts like diabetes, chronic kidney disease with heavy proteinuria, cardiac failure, myocardial infarction, high blood pressure, vascular aging. MR has been reported to be expressed in non-classical targets like podocytes or mesangial cells during pathological situations only like type I diabetes in the rat and in spontaneous hypertensive rats with metabolic syndrome (SHR/cp rat), both conditions in which MR expression is clearly stimulated in podocytes in vivo.
Renal pathophysiological consequences of MR activation in vivo.

The pathophysiological consequences of MR activation in the kidney have been described in experiments done with aldosterone infusion, with suppression of aldosterone synthesis after adrenalectomy, and with pharmacological MR antagonism. Implication of renal MR activation has been reported in hypertensive nephropathy, chronic renal disease with glomerulosclerosis and proteinuria associated to subtotal nephron reduction and experimental models of proliferative glomerulopathies, nephritic syndrome and lupus nephropathy. The role of Aldosterone and/or MR activation in the diabetic nephropathy has also been demonstrated in experimental models with type I diabetes (streptozotocin-induced) or in type II diabetes (db/db mice). Very recently, aldosterone and/or MR activation have been proposed as deleterious factors in Ciclosporine A nephrotoxicity upon kidney transplantation.

Mineralocorticoids and kidney diseases: clinical evidence.

The use of MR antagonists is supported by aldosterone “breakthrough” reported in ACE-I and/or ARB-treated patients, where aldosterone breakthrough is defined by an increase in circulating aldosterone levels after the initiation of a RAAS blockade as compared to the values before initiation of the treatment, despite blockade of the effects of angiotensin II. Several studies with positive outcomes have been performed in humans not only in the diabetic nephropathy and other proteinuric nephropathies, but also in End Stage Renal Disease patients and in children. In most of the clinical studies the primary endpoint was reduction in proteinuria and/or albuminuria. The mechanisms underlying these beneficial effects remain to be explored but could combine effects on podocyte injury, renal hemodynamics, reduction of oxidative stress and inflammatory activation. A more general effect on the cardiovascular system, with secondary implications on the progression of renal injury may also be considered. The possibility that the beneficial effects of MR antagonism may not require an effect on proteinuria per se is raised by studies in anuric End Stage Renal Disease patients showing cardiovascular effects of MR antagonists. Those performed in anuric patients are informative on the effects of MR antagonists that are independent from their action on the renal epithelium or the glomerulus.

Adverse effects in using MR antagonists.

The adverse effects in MR antagonists could be divided in ionic effects (hyperkalemia and salt depletion related to the diuretic effect) and anti-androgenic effects (gynecomastia, disorders of the menstrual cycle, etc., related to the non-specific androgen receptor blockade). The use of MR antagonists is classically not recommended in CKD patients, because of the concerns about hyperkalemia, which often occurs when multiple RAAS blockers are used. Several authors suggest that the MR antagonists are not used often enough in heart failure, based on comparisons to the inclusion criteria for the RALES and EPHEUS studies. They also suggest that the biological follow-up (monitoring of serum creatinine and potassium) is not optimal. Several authors state that the safety issues related to hyperkalemia may be overshadowed by the potential beneficial effects of MRBs in patients with CKD. Indeed, the risks associated with hypokalemia appear...
to be more consequential than the risks associated with hyperkalemia in patients with CKD. Controlling hyperkalemia during RAAS blockade include dietary restriction, increased colonic secretion of potassium and the use of adjunctive diuretic therapy\textsuperscript{16}.

**Conclusion.**

The pathophysiological implication of aldosterone and the mineralocorticoid receptor has been demonstrated *ex vivo* in cell culture and *in vivo* in experimental animal models with kidney damages such as diabetic and hypertensive kidney nephropathies, chronic kidney disease and glomerulopathies. The benefits of pharmacological mineralocorticoid receptor blockade in this clinical setting have been demonstrated in human patients and should stimulate large clinical trial to decipher benefits and risks of MR antagonisms in kidney diseases of various origins.

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Q & A - Cardio-Renal Syndrome, what is behind it?

Chair Clarke E. Atkins (CA) - USA
DVM, DipACVIM (Internal medicine and Cardiology)

Panel:

Rebecca L. STEPIEN (RS)
DVM, MS, DipACVIM (Cardiology) - USA

Claudio RONCO (CR)
MD - Italy

Frédéric JAISSE (FJ)
MD, PhD - France

Question (Michele Borgarelli) — We have a study that has been published recently in veterinary medicine, the Quest trial*. What was actually surprising was that the patients with the higher creatinine had better outcomes than the patient that had lower creatinine. I would like to know your opinion about that.

Answer (FJ) — We are not sure, it may be that these patients had higher muscle mass or were in better shape.

Answer (CR) — We should ask whether the creatinine levels were corrected for levels of hydration, and whether they are true values? We don’t really know whether these creatinine levels were reflective of a true creatinine increase. We know that in humans, the level of fluid overload correlates with outcome. So theoretically speaking, one explanation could be that you have a level of fluid overload that relatively decreased levels of creatinine. It may indeed affect outcome.

Question to CR (Mark Oyama) — What role does ultrafiltration play in the management of heart failure? Does it blunt the development of cardiorenal syndrome?

Answer (CR) — There are two sides to ultrafiltration. If treated correctly it removes the level of cough and improves renal function. The question is how much fluid to remove and you need to check bioimpedance and blood volume. However, cardiologists tend to see ultrafiltration as the last hope when it is better used as an elective rather than rescue treatment. Keeping the patient ‘dry’ will improve the quality of life and reduce diuretic usage.

Q & A - Cardio-Renal Syndrome, what is behind it?

Comment (Jonathan Elliott) — It is our observation that, when you look at dogs in heart failure, few cases go on to develop kidney damage. Dogs with DMVD are very common but kidney disease is only 10-20% of the level that we see in cats. Conversely there are lots of cats that have kidney failure but few progress to heart failure. Maybe it’s because we euthanise them before they develop cardiorenal syndrome or cardiovascular complications, and we are not doing enough renal replacement therapy to prolong the lives of those animals and see the really chronic cases.

Answer (RS) — It is true that we don’t prolong lives much when our patients get heart failure or kidney failure, so perhaps that’s why we are not seeing cardiorenal syndrome. Maybe we simply euthanise dogs and cats before their condition becomes very chronic.

Comment (David G. Warnock, MD, Director of the Division of Nephrology, Professor of Medicine and Physiology at the University of Alabama, Birmingham, USA) — The clinical précis is that frusemide is toxic in intensive care units. Maybe it is more of a marker for a mismatch between circulatory volume and heart/kidney function? In comparison, when you perform ultrafiltration, the patient is very carefully monitored, so fluid changes happen precisely, compared with the blunt effects of diuretics.

Answer (RS) — It is sobering to see how embryonic we vets are using drugs to treat heart failure. We just tend to pull out the ‘biggest club’, when we could have gotten better results with something lower.

Answer (CR) — There are two sides to diuretics, just as the god Janus. On the one hand they remove congestion but on the other hand interfere with the delicate regulation of an organ, i.e. the kidneys. It is essential to monitor the five Bs: blood pressure, biomarkers, bioimpedance, blood volume and the last one, which is often neglected in cardiology, fluid balance.

Question (Chair) — In diuresis, we are using a hammer and it is easy to cast aspersions on diuretic treatment?

Answer (CR) — There was a commentary in the American Journal of Kidney Disease* on the difference between high versus low dose diuretics, and continuous versus intermittent treatment. The reality is that every patient is different but gentle low dose therapy is generally better than aggressive high dose. But, in the general population there is no final effect on outcome, and the recommendation is to treat patients according to the best possible approach.

Question (Bertram Pitt) – There is the question of diuretic resistance. For instance, if we use very high doses of spironolactone (100-200 mg), we can overcome this resistance.

**Answer (CR)** — I don’t believe that there is such a thing as diuretic resistance. Instead we need to make the right mix of drugs to improve diuresis. If the patient still isn’t making urine despite these drugs, we need to re-analyse the patient.

**Question to FJ (Chair)** — The mineralocorticoid receptor is more ubiquitous than believed and there are benefits of blockade even if there is no change in proteinuria? I wonder if you would comment about your goals in terms of treating a proteinuric patient and if you’re unable to make proteinuria go away, how are you able to handle those patients?

**Answer (FJ)** — Clearly, the proteinuria decreases when you treat with mineralocorticoid receptor antagonists. What is not that clear right now is whether this improves renal function. It’s not that clear from the small clinical trials that have been done. I think the endpoint is indeed to improve renal function and we need larger and also longer trials to see whether it will really improve renal function.

**Question to FJ (CR)** — Can you comment on the use of ACE inhibitors and mineralocorticoid receptor blockers, used simultaneously or separately?

**Answer (FJ)** — Their effects have not been studied concurrently either in animal models of kidney disease or in human patients. There is a lot to be done to know what works, how it works and whether, like in the heart, there is a benefit to have a combination or not.

**Question to FJ (CR)** — On the subject of mineralocorticoid receptor pleotropic effects, are these similar to the vitamin D receptor? Do we need a more holistic approach to therapy?

**Answer (FJ)** — We still have a lot to learn on these receptors.

**Question to CR (Adrian Boswood)** — There have been studies of sodium intake in humans with heart failure and apparent diuretic resistance. The Italian group recommends an increase in sodium intake or even the use of hypertonic saline to increase free water excretion. What do you feel?

**Answer (CR)** — I am sceptical. In patients whose sodium pool is increased I want to normalise sodium concentrations and the volume pool in the body. So the idea of increasing sodium is conflicting to the physiology. I am concerned and would like to see more information.

**Comment (AB)** — I agree that the findings are counterintuitive but the results are striking.

**Answer (CR)** — I don’t have an explanation and want to see more data.
Question to CR (Bertram Pitt) — Can you comment on the failure of vasopressin II antagonists? What went wrong?

Answer (CR) — The rationale for their effect was there, as was the mechanism. The results were not as expected. One possible explanation lies in the enrolment; maybe the trial population was not reflective of the true population in clinical practice. For example, renal failure patients were excluded in trials of patients with heart failure. We need to look for more results from prospective studies as this is an important molecule. The mechanisms are likely complex and it is possible that the kidneys are smarter than we are!
CHAIRMEN FOR SCIENTIFIC PROGRAM

JONATHAN ELLIOTT
MA, VetMB, PhD, CertSAC, DipECVPT, MRCVS
Vice Principal – Research
Professor of Veterinary Clinical Pharmacology
Royal Veterinary College
London, UK
Contact: jelliott@rvc.ac

JENS HÄGGSTRÖM
DVM, PhD, DipECVIM (Cardiology)
Professor Internal Medicine
Department of Clinical Studies, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences
Uppsala, Sweden
Contact: jens.haggstrom@kv.slu.se

CLARKE E. ATKINS
DVM, DipACVIM (Internal medicine and Cardiology)
Jane Lewis Seaks Distinguished Professorship of Companion Animal Medicine
Department of Clinical Sciences, College of Veterinary Medicine North Carolina State University.
Raleigh, North Carolina. USA
Contact: ceatkins@ncsu.edu
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 lectureship in Veterinary Pharmacology at the Royal Veterinary College and developed research interests in feline kidney disease and hypertension, canine mitral valve disease and Equine Laminitis. He was awarded the Pfizer Academic Award in 1998 and the BSAVA Amoros 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.

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

Jens Häggström is a Professor in the Department of Clinical Studies, Faculty of Veterinary Medicine and Animal Science. His main interests in clinical research concern heart disease in dogs and cats (MMVD, DCM and cardiomyopathy in cats), diagnostic techniques, pathophysiology and therapy of heart failure, and genetic risk factors for heart disease in dogs and cats.

Jens Häggström was born and brought up in Uppsala, Sweden, where he also completed his basic veterinary training in 1990. He achieved his Doctorate degree in 1996 with a thesis concerning MMVD in dogs. In 2000, Jens became an Associate Professor in Uppsala and in 2003 became full Professor in Internal Medicine. He earned his status as Diplomate of the European College of Veterinary Internal Medicine in 1998 and served in different Committees in the period 1999-2006 and as Chairman of the Cardiology subspeciality during 2003-2006. Since 2009 he is a co-lecturer of the European School of Advanced Veterinary Studies for the Cardiology program.

Professor Häggström is also author and co-author of a large number original papers in internationally distributed peer-viewed journals, congress abstracts and textbook chapters. He resides in Uppsala with his family, wife and 2 sons, and enjoys running, racket sports, golf and skiing during his spare time.

Clarke Atkins, DVM, Professor of Medicine and Cardiology at North Carolina State University is a 1972 graduate of the University of California, Davis and an Angell Memorial Animal Hospital intern. He is board-certified by the ACVIM in internal medicine and in cardiology.

Dr. Atkins is known for his research and teaching in small animal cardiology, he is the 2004 Norden-Award recipient for excellence in teaching, and was recently named the Jane Lewis Seaks Distinguished Professor.

He is the author of over 150 publications and his research involves canine and feline heartworm disease and pharmacologic therapies of cardiac disease in dogs, cats and horses.
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