

The use of cardiac biomarkers in veterinary medicine: the equine perspective

*Diergeneeskundig gebruik van cardiogene biomarkers :
focus op het gebruik bij paarden*

N. Van Der Vekens, A. Decloedt, D. De Clercq, T. Verheyen, G. van Loon

Department of Large Animal Internal Medicine
Faculty of Veterinary Medicine, Ghent University
Salisburylaan 133, 9820 Merelbeke, Belgium

Nicky.VanDerVekens@Ugent.be

ABSTRACT

In human medicine, cardiac biomarkers, such as natriuretic peptides and troponins, are routinely used for the diagnosis, prognosis and monitoring of heart diseases. Similarly, these biomarkers are determined in small animals to differentiate non-cardiac from cardiac diseases. Knowledge about these biomarkers in horses is limited and requires further investigation. The first equine studies about atrial natriuretic peptide (ANP) and N-terminal ANP (NT-proANP) are promising, and show a clear correlation with atrial dimension size. Equine brain natriuretic peptides assays are still unavailable. The troponins, in particular troponin I, have been more extensively studied in horses, and their use for the diagnosis of myocardial damage has been fully demonstrated. They have replaced the less specific lactate dehydrogenase and creatine kinase iso-enzymes, which makes the use of the last mentioned no longer legitimate. A final possible equine biomarker is aldosterone. Reference values in horses have been established. However, in only one study, a correlation between aldosterone and cardiac disease has been reported.

SAMENVATTING

Cardiale biomarkers, zoals natriuretische peptiden en troponinen, worden reeds routinematig in de humane geneeskunde gebruikt voor de diagnose, prognose en monitoring van hartaandoeningen. Hoewel ze ook in de kleine huisdierensector regelmatig bepaald worden om cardiologische en niet-cardiologische aandoeningen van elkaar te onderscheiden, is de literatuur omtrent het gebruik bij paarden beperkt. Bij paarden werd reeds een significante correlatie aangetoond tussen het 'atrial natriuretic peptide' (ANP), N-terminal ANP (NT-proANP) en de voorkamergrootte. Specifieke equine testen voor 'brain natriuretic peptide' (BNP) of 'N-terminal BNP' (NT-proBNP) bepaling ontbreken echter nog steeds. Troponinen (vooral troponine I) zijn meer uitgebreid bestudeerd bij het paard. Ze worden reeds gebruikt voor de diagnose van myocardschade en vervangen de minder specifieke lactaatdehydrogenase en creatinekinase iso-enzymen, waardoor het gebruik van deze laatste niet meer gerechtvaardigd is. Een laatste mogelijk belangrijke biomarker is aldosteron, maar aangezien slechts één studie bij paarden een correlatie aangetoond heeft tussen de aldosteronconcentratie en hartaandoeningen is uitgebreider onderzoek nodig.

INTRODUCTION

Over the last two decades, different hormones, enzymes and other proteins have appeared as markers for cardiac problems. In human medicine, one of the most important applications of these cardiac biomarkers is to diagnose acute myocardial infarction in patients with chest pain (Christenson et al., 1998). A number of these proteins also play an important role in assessing prognosis in cardiac patients, and the combination of these individual biomarkers will probably be used in the future as guidance for therapy; the so-called multi-marker strategies (Böhm et al., 2011).

Similar to human medicine, a correlation between the cardiac biomarkers and the prognosis of cardiac disease has been found in animals (Greco et al., 2003). The recent literature describes the utility of these peptides for distinguishing cardiac from non-cardiac diseases in small animals (Fox et al., 2009). These markers have also found their way in equine cardiology for assessing the severity of heart valve diseases (Trachsel et al., 2011). This article reviews the most important cardiac biomarkers and their use in veterinary, and more particularly, equine medicine. Although there are many unresolved questions about the different biomarkers, their future use to screen cardiac diseases is realistic.

Table 1 summarizes the main cardiac biomarkers used in human medicine. The troponins and natriuretic peptides are mostly utilized in daily practice, but also aldosterone, a hormone of the renin – angiotensin-aldosterone system (RAAS) can be measured to evaluate the cardiac state.

THE NATRIURETIC PEPTIDES, MARKERS OF MYOCYTE STRESS

Physiology and structure

In mammals, there are three main natriuretic peptides: the atrial natriuretic peptides (ANP), the brain natriuretic peptides (BNP) and the C-type natriuretic peptide (CNP). Their main goal is fluid homeostasis. They are released in case of increased intravascular volume and thus atrial or ventricular stretch in an attempt to decrease cardiac overload by diuresis, natriuresis and vasodilatation. Since CNP only causes mild diuresis and natriuresis, only atrial and brain natriuretic peptides are used in clinical practice to detect cardiac disease (Goetze et al., 2003; Takei et al., 2011).

The atrial natriuretic peptides are mostly stored as

pro-hormones (proANP₁₋₁₂₆) in numerous granules in the atrial cardiomyocytes. However, they are also present in other organs, such as the pituitary, lungs, hypothalamus and kidneys (Kokkonen et al., 2002; Hayek and Nemer, 2010). In response to atrial stretch, the pro-ANP₁₋₁₂₆ molecule is split into an inactive NH₂-terminal peptide (NT-proANP₁₋₉₈) and a biologically active (COOH-terminal) ANP₉₉₋₁₂₆ molecule, which are both released into the blood stream (Figure 1). After release, ANP has a short half-life of about 2-5 minutes in humans. Since the half-life of NT-proANP is longer (55-60 minutes), NT-proANP can be used to assess endogenous secretion of ANP (Thibault et al., 1987; Ruskoaho, 1992; Kokkonen et al., 2002). The structure of the ANP-molecules is well conserved, and 100 % homology exists between the equine and human ANP (Table 2), which implies that human tests can be used to measure the ANP concentration in equine blood (Richter et al., 1998). NT-proANP is more species-specific with only 80-90% homology between the equine and the human NT-proANP peptide (Kokkonen, 2002).

Brain natriuretic peptide was first discovered in the porcine brain. It is currently often called the B-type natriuretic peptide because its main production site is ac-

Table 1. Summary of the most important cardiac biomarkers in human medicine. Biomarkers in bold are discussed more in detail below (adapted from Braunwald, 2008).

Markers of myocyte stress	Brain Natriuretic Peptide (BNP) N-Terminal Pro Brain Natriuretic Peptide (NT-proBNP) Atrial Natriuretic Peptide (ANP) N-Terminal Pro Atrial Natriuretic Peptide (NT-proANP) Midregional proadrenomedullin ST2
Markers of myocyte injury	Troponin T Troponin I Creatine kinase MB fraction Lactate dehydrogenase Myosin light chain kinase I Heart type fatty acid protein
Neurohormones	Aldosterone Norepinephrine Renin Angiotensin II Arginine vasopressin (Big) endotheline 1
Markers of remodeling	Matrix metalloproteinases Tissue inhibitors of matrix metalloproteinases Collagen propeptides
Markers of inflammation	C-Reactive Protein (CRP) Interleukins 1, 6 and 19 Tumor Necrosis Factor alpha (TNF- α) Fas (APO-1)
Indirect markers of oxidative stress	Oxidized low density lipoproteins Myeloperoxidase Urinary biopyrrins Urinary and plasma isoprostanes Plasma malondialdehyde

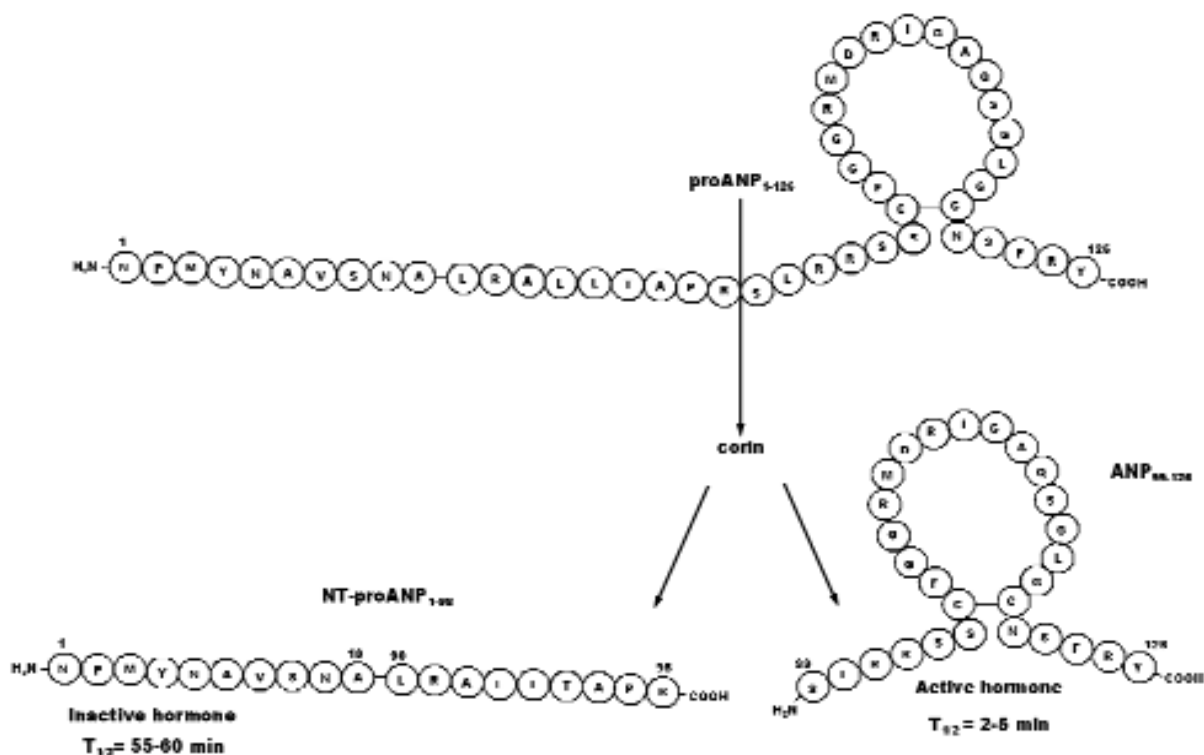


Figure 1. Enzymatic cleavage of proANP by corin into NT-proANP and ANP (T_{1/2} = half-life; min = minutes).

tually the heart. In contrast to atrial natriuretic peptides, BNP is more related to ventricular pathological changes. In healthy individuals, BNP gene expression occurs mainly in the atria. However, an up-regulation in the ventricles can be found in people with heart failure (Goetze, 2004a; Hall, 2005). Similar to the atrial natriuretic peptides, activation of proBNP₁₋₁₀₈ results in cleavage of this molecule into a biologically inactive (NT-proBNP₁₋₇₆) and an active component (BNP₇₇₋₁₀₈) (Hall, 2005). Both BNP (12.1 min) and NT-proBNP (120 min) have a longer half-life than the atrial natriuretic peptides, which makes them better targets for diagnostic blood testing (Kemperman et al., 2004; Kimura et al., 2007).

In comparison with ANP, the B-type peptides are genetically very variable between species (Takei et al., 2011) (Table 2). Therefore, a human test cannot be used in veterinary practice. A specific test is available for small animals (Cardiopet proBNP, IDEXX Vet Med Lab, Hoofddorp, the Netherlands). However, an equine test is not available yet. Since there is a gross similarity (±90% homology) between the equine and the porcine BNP peptides, a porcine test could potentially be used to measure the BNP concentration in equine blood (Table 2). The homology between these molecules was first suggested in 1995, when antibodies against porcine BNP were utilized for the detection of BNP-like molecules in the equine atria (Mifune et al., 1995).

Table 2. Comparison of human, equine, porcine, canine and feline amino-acids sequences of atrial and brain natriuretic peptides. In contrast to ANP, BNP is very variable between species. The equine B-type natriuretic peptide has a greater resemblance to the porcine form than the human BNP-molecule. The red-colored amino-acids are species specific (adapted from Richter et al., 1998; Goetze, 2004; Takei et al., 2011).

Atrial natriuretic peptide															
Human	S	L	R	R	S	S	C	F	G	G	R	M	D	R	I
Equine	S	L	R	R	S	S	C	F	G	G	R	M	D	R	I
Porcine	S	L	R	R	S	S	C	F	G	G	R	M	D	R	I
Canine	S	L	R	R	S	S	C	F	G	G	R	M	D	R	I
Feline	S	L	R	R	S	S	C	F	G	G	R	M	D	R	I
	G	A	Q	S	G	L	G	C	N	S	F	R	Y		
Brain natriuretic peptide															
Human	S	P	K	M	V	Q	G	S	G	C	F	G	R	K	M
Equine	S	P	K	M	M	R	N	S	G	C	F	G	R	R	L
Porcine	S	P	K	T	M	R	D	S	G	C	F	G	R	R	L
Canine	S	P	K	M	M	H	K	S	G	C	F	G	R	R	L
Feline	S	S	K	M	M	R	D	S	R	C	F	G	R	R	L
	D	R	I	G	S	L	S	G	L	G	C	N	V	L	R
	R	L	R	L	R	R	Y	K	H						

The use of natriuretic peptides in human and small animal veterinary medicine

In human medicine, the brain natriuretic peptides are frequently used for the diagnosis, prognosis and therapy monitoring in patients with heart failure. Even though the atrial natriuretic peptides were discovered first, nowadays, the B-types are used more often in clinical practice, because of their longer half-life (Woodard and Rosado, 2007). Not only are levels of BNP and NT-proBNP elevated in cases of heart failure, also non-cardiac pathologies, such as acute pulmonary embolism, pulmonary hypertension, sepsis or hyperthyroidism may influence their concentration (Felker et al., 2006).

Adjusted BNP and NT-proBNP levels are used in emergency departments to differentiate between non-cardiac and cardiac causes of dyspnea (Maisel et al., 2002; Rogers et al., 2009). Similarly, a species-specific NT-proBNP test allowed to distinguish acute respiratory problems caused by heart failure from non-cardiac causes in small animals (Fox et al., 2009; Ettinger et al., 2012). In human medicine, the brain natriuretic peptides are also used for the detection of myocardial ischemia in patients with acute coronary syndrome. Compared with the conventional markers (e.g. troponin I), the NT-proBNP molecules rise much higher in an earlier phase, making them more suitable for early diagnosis (Tsai et al., 2010). Myocardial ischemia is rare in small animals, but in resemblance to humans a significant increase in NT-proBNP concentration has been found in a canine model of myocardial ischemia (Hori et al., 2012). A much more common disease in small animals, especially in cats, is hypertrophic cardiomyopathy (HCM). Investigation into the use of NT-proBNP elevation as screening factor for this disease revealed that it can only be used for the detection of cats with severe HCM: the NT-proBNP concentration in cats with moderate or mild HCM is not significantly different from normal individuals (Hsu et al., 2009).

As mentioned above, the use of atrial natriuretic peptides in human clinical practice is limited (Saito, 2010). Recently, the diagnostic value of a fragment of the proANP molecule (mid-region proANP) for heart failure has been recognized (Ghosh and Haddad, 2011), and a multi-marker strategy is emerging. Because of the species-similarity of the ANP-molecules, no specific veterinary test had to be developed and a human radio-immunoassay (RIA) has been validated in dogs as predicting factor for survival (Greco et al., 2003). An ANP cut-off value of 25 pg/mL shows a high specificity and sensitivity for identifying dogs with heart diseases (Hori et al., 2011). In this study, a positive correlation between the ANP concentration and the left atrial-to-aortic root diameter could be demonstrated, which confirms that natriuretic peptides are released in cases of atrial stretch (Shionoria-ANP, Shionogi Co, Osaka, Japan). A similar correlation has been found in cats with cardiomyopathy (Hori et al., 2008).

The (possible) use of natriuretic peptides in equine veterinary medicine

As stated above, no specific equine BNP or NT-proBNP test is yet available and no equine specific half-life has been determined. Therefore, equine biomarker studies are limited to the measurement of atrial natriuretic peptides with human assays. Gehlen et al. (2007) and Trachsel et al. (2011) have already determined normal values for horses (15.6–26.4 pg/mL and 7.2–34 pg/mL) using two different human radioimmunoassays (RIA S2011 Peninsula Laboratories, San Carlos, USA; RIA RK-005-06 ANP- α , Phoenix Pharmaceuticals Inc.), and found a positive correlation between the ANP concentration and left atrial enlargement.

In contrast to human and small animal veterinary medicine, the extra-cardiac influences on natriuretic peptides have been poorly investigated in horses. An important effect of exercise has been described (Kokkonen, 2002). In this study, the maximum ANP and NT-proANP values were reached respectively 5 and 30 minutes after exercise, and both peptides remained high until 60 minutes post-exercise. In anticipation of further studies, samples should therefore not be taken after exercise. Sample handling and stability of the molecules should also be taken into account. Stability studies conducted in human medicine have not revealed any clear standard sampling technique (Nelesen et al., 1992; Buckley et al., 1999; Zolty et al., 2008). Since no stability studies have been established for equine ANP and NT-proANP, human data are often extrapolated (Gehlen et al., 2007; Trachsel et al., 2011). However, this should always be done with caution, since species differences do exist. Therefore, details of sample handling and storage are imperative for the correct interpretation of the results. Reliable results can be obtained if aprotinin (a proteinase-inhibitor) is added to blood EDTA (ethylenediaminetetraacetic acid) samples and centrifuged at 4°C within 30 minutes. Plasma storage at -80°C is preferred until the assay is performed. Since significant ANP degradation has been shown in samples stored at -80°C for seven days, plasma samples should be assayed immediately or within a few days (Nelesen et al., 1992).

THE TROPONINS, MARKERS OF MYOCYTE INJURY

Physiology and structure

Troponins are part of the contractile apparatus of muscle tissue and can be found both in skeletal and cardiac muscles. They form a complex consisting of three interacting proteins: troponin I, T and C (Sandersen et al., 2008). Expression of cardiac isoforms with unique contractile properties is found in the heart, and is normally not present in adult skeletal muscle (Parmacek and Solaro, 2004). However, cardiac troponin T expression has been described in regenerating skeletal muscles in patients with skeletal myopathies (Maynard

et al., 2000). Troponins are mainly bound to myofibrils and only 6-8 % of cardiac troponin T (cTnT) and 2.8-4.1 % of cardiac troponin I (cTnI) can be found in the cytosol. Damaging cardiac myocytes primarily causes release of the cytosolic troponin pool. At a slower rate, the structurally bound troponins are released, which sustains the troponin elevation (Maynard et al., 2000). The concentrations begin to rise 4-8 hours after ischemic injury until a peak is found after 12-24 hours. The levels of cTnT and cTnI remain raised for 10-14 days and 7-10 days, respectively.

The use of cardiac troponins in human and small animal veterinary medicine

The primary role of cardiac troponin testing in humans is the diagnosis of acute myocardial infarction (AMI). In the last decade, troponins have replaced the less sensitive lactate dehydrogenase (LDH) and creatine kinase-myocardial band (CK-MB) isoenzymes. Not only are troponins more specific, their elevations remain longer, making them (besides NT-proBNP) an excellent biomarker for the detection of AMI (Maynard et al., 2000; Wells and Sleeper, 2008). Currently, cTnT is mostly used in human clinical practice. However, cTnI is comparable in diagnostic and prognostic efficacy. Some authors even claim cTnI superiority in cases of renal impairment and skeletal muscle damage, since cTnT specificity for cardiac muscle may not be absolute (Maynard et al., 2000). The use of the newer fourth generation cTnT assays could possibly limit cross reactivity in patients with renal disease. However, primary skeletal muscle disease could still cause false positive results (Jaffe et al., 2011).

In contrast to natriuretic peptides, the cardiac troponin structure is very conserved across species, which indicates that human assays can be utilized in different animals (O'Brien et al., 1998). In dogs, reference values for cTnI have already been established using a human immunoassay (Acces Accu TnI Assay Beckman Coulter, Inc., Fullerton, CA) and a human point-of-care cTnI analyzer (i-STAT 1 handheld clinical analyzer, Heska Corporation, Loveland, CO) (Oyama and Sisson, 2004; Payne et al., 2011). An increase in cTnI has been reported in dogs with cardiomyopathy, subvalvular aortic stenosis, mitral valve disease and cardiac causes of respiratory distress (Oyama and Sisson, 2004; Linklater et al., 2007; Bakirel and Gunes, 2009). Similar studies in cats have described the rise of cTnI in cats with cardiac dyspnea (Herndon et al., 2008). In comparison with cTnI, cardiac troponin T seems less sensitive for the diagnosis of cardiac diseases in dogs. The exact reason is still unknown, but Linklater et al. (2007) suggested that the smaller size of cTnI (molecular weight: 22 000 Da) could lead to an easier release of this molecule from the tropomyosin complex than the bigger cTnT protein (molecular weight: 40 000 Da). This size difference could also explain the higher values of cTnT in patients with renal failure, since the renal clearance of the large cTnT is more compromised (Payne et al., 2011).

The use of troponins in equine veterinary medicine

Currently, cardiac troponin I is the only cardiac biomarker which has been successfully used in equine veterinary practice for the evaluation of myocardial damage. Reference values have been established with different assays, and recently, the equine cTnI half-time has been determined.

A mean serum cTnI concentration of 0.047 ± 0.085 ng/mL has been reported in Thoroughbred horses with the Dimension Heterogeneous Immunoassay Module (Siemens, Newark, DE) (Phillips et al., 2003). The same breed was studied by Begg et al. (2006), in which serum and plasma cTnI concentrations under the detection limit (<0.15 ng/mL) were found in all healthy horses using a commercial assay (ADVIA Centaur cTnI assay, Bayer Corporation, Pittsburg, PA, USA). In a more recent study, a point-of-care analyzer (i-STAT 1, Heska Corp, Loveland, Colorado) was compared with a Beckman Access Immunoassay (Ohio State University Medical Center Reference Laboratory, Columbus, Ohio), achieving plasma concentrations from 0.0-0.06 ng/mL for normal healthy horses of different breeds (Kraus et al., 2010).

One should keep in mind that reference values can vary among different assays and laboratories. Thus, a sample from a horse with cardiac problems should always be compared with assay-specific reference values. As the half-life is 0.47h in horses, a persistent high cTnI value correlates with ongoing myocardial damage (Kraus et al., 2011). This short half-life also influences the optimal time for determining maximal elevations of cTnI. In theory, the optimal sampling time is 1-2h after a suspected maximal increase of cTnI. Similar to ANP, exercise results in an increase of cTnI (Nostell and Högström, 2008). Not only the time of sampling but also sample handling can affect results. Since sample stability depends on the assay antibody configuration, sample type and handling should be performed according to the manufacturer's instructions (Tate and Panthegini, 2008). Abbot Laboratories for example recommends centrifugation of serum samples (after complete clot formation) at 2500-3000 g for 10 minutes if testing is delayed for more than eight hours. According to these instructions, samples can be stored up to 72 hours at 2-8°C and up to 30 days when frozen at a maximum temperature of -10°C (Manual of the Architect STAT troponin I system, Abbott Laboratories, Abbott Park, Illinois).

If all these factors are taken into consideration, cTnI concentration can be used in daily equine practice for the detection of myocardial damage. In the literature, the use of cTnI has been increasingly described in horses, and retrospective studies show positive associations between an increase in cTnI and the degree of cardiac damage at necropsy (Divers et al., 2009; Kraus et al., 2010). In some case reports, this increase is extremely high. In a study by Schwarzwald et al. (2003), a cTnI concentration of 404 ng/mL was measured (ADVIA Centaur cTnI assay, Bayer Corporation, Pitts-

burgh PA) in a horse with multiform ventricular tachycardia. An even higher value (816 ng/mL) has been reported in a horse with lasalocid intoxication (Decloedt et al., 2012).

In contrast to human medicine, the cardiac troponin T molecule has a lower diagnostic value in horses. In one study, a human quantitative (Enzym Linked Immunosorbent Assay from Boehringer Mannheim) and qualitative assay (Trop T Sensitive Rapid Assay, Roche Diagnostics, Mannheim, Germany) were used (Gehlen et al., 2006). Similar to cTnI, all of the healthy horses had normal values under the limit of detection (<0.06 ng/mL). A significantly higher cTnT value was found in horses with severe valve insufficiencies and cardiac dilatation. No significant increase could be demonstrated in horses with moderate valve insufficiencies without cardiac dilatation. Another important result was the surprisingly high cTnT value in 11 horses with atypical myopathy (Gehlen et al., 2006). Since atypical myopathy causes extreme muscle damage, a cross reaction between myocardial and skeletal cTnT could explain these high values. However, cardiac damage with elevation of cTnI has also been described in patients with atypical myopathy (Verheyen et al., 2012). A high cTnT value was also found in a horse with renal failure. Although it concerned only one horse, the case highlights the importance of renal clearance when interpreting cTnT elevations. Cardiac troponin T has also been measured in horses with atrial fibrillation before and after transvenous electrical cardioversion (McGurrin et al., 2008). Since all concentrations were below the limit of detection, no increase could be observed.

ALDOSTERONE

Physiology and structure

The normal physiology of the renin-angiotensin-aldosterone system (RAAS) is shown in Figure 2. Its main function is the monitoring of blood flow, pressure, oxygen and Na⁺ concentrations (Muñoz et al., 2010). In response to a decrease in one of these parameters, renin is released from the juxtaglomerular apparatus in the kidney, which transforms the circulating angiotensinogen into the biologically inert angiotensin I. Subsequently, angiotensin I is cleaved by the angiotensin converting enzyme (ACE) into the biologically active angiotensin II. This has various effects, including aldosterone secretion, which increases blood flow and blood pressure by stimulating Na⁺ reabsorption and vasoconstriction (Weber, 2001).

Aldosterone release in horses with cardiac problems

In case of cardiac failure, the decreased arterial filling activates the RAAS, which raises the aldosterone concentration. In response to this aldosterone release, water retention increases for the maintenance of the

blood flow. Unfortunately, this volume expansion exacerbates diastolic wall stress and increases afterload, which further deteriorates the already reduced ventricular function (Muñoz et al., 2010).

The correlation between aldosterone concentration and cardiac disease has already been observed in humans and dogs (Böhm et al., 2011; Haggstrom et al., 1997). Unfortunately, aldosterone failed as guidance molecule for the treatment of heart failure. Only one study has been performed in horses with cardiac disease, in which a significant difference in aldosterone concentration between healthy horses and horses with left atrial and left ventricular enlargement was found (Gehlen et al., 2008). However, no specific cut-off value for severity of heart valve diseases was derived, emphasizing the need for further research.

Besides cardiac disease, many non-cardiac factors, such as sodium intake, exercise and circadian rhythm, influence aldosterone concentration (Guthrie et al., 1982; Harris, 1993). In humans, a diurnal aldosterone rhythm has been determined with maximal and minimal values round 08:00h am and 02:00h am, respectively (Chiang et al., 1994). Such a diurnal variation was

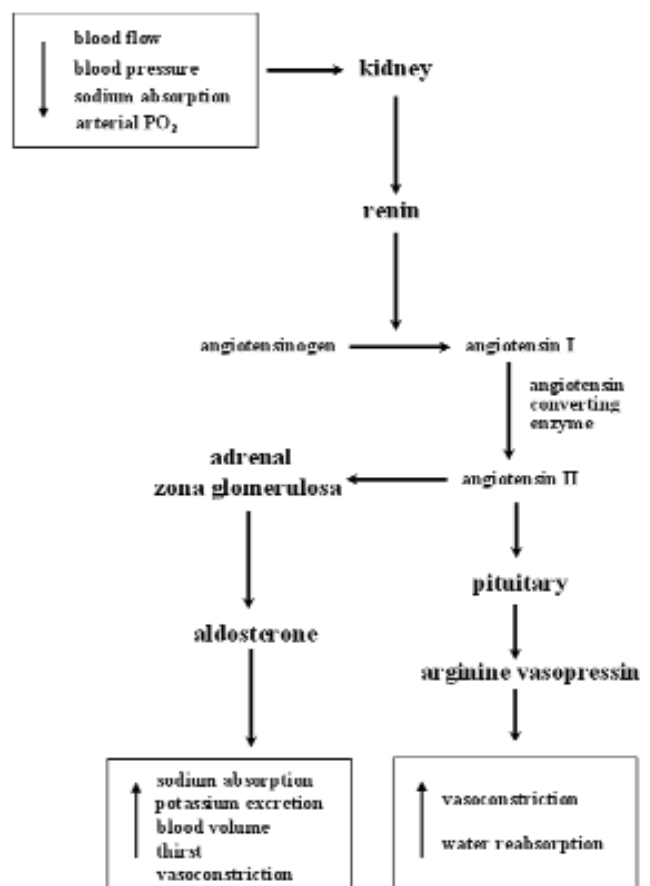


Figure 2. Scheme of the normal renin-angiotensin-aldosterone-system (RAAS). Low blood flow, blood pressure, sodium absorption or arterial blood PO₂ cause activation of the RAAS with release of renin and finally the production of aldosterone and vasopressin (adapted from Muñoz et al., 2010).

also noted in a study in horses by Jansson et Dahlborn, 1999. However, the highest values were reported during the evening and in the night. Human and equine aldosterone are identical, thus a human radio-immunoassay can be used to measure aldosterone concentrations in horses (Harris, 1993). Besides sampling time, sample handling and storage should also taken into account in clinical practice. Aldosterone plasma and serum samples were stable for 120h at 4°C in one study (Evans et al., 2001). However, individual laboratory recommendations should always be followed.

CONCLUSION

Cardiac biomarkers are intensively used in human and small animal veterinary medicine for the diagnosis and prognosis of heart diseases. This trend will further develop in equine medicine and stimulates equine specific studies and the development of assays. Currently, only cardiac troponin I can be used in equine clinical practice for the diagnosis of myocardial damage, thereby replacing the less specific LDH and CK-MB iso-enzymes. In the future, natriuretic peptides will become more important to estimate the diagnosis and prognosis of heart diseases in horses.

LITERATURE

- Bakirel U., Gunes S. (2009). Value of cardiac markers in dogs with chronic mitral valve disease. *Acta Veterinaria* 59, 223-229.
- Begg L.M., Hoffmann K.L., Begg A.P. (2006). Serum and plasma cardiac troponin I concentrations in clinically normal Thoroughbreds in training in Australia. *Australian Veterinary Journal* 84, 336-337.
- Böhm M., Voors A., Ketelslegers J.-M., Schirmer S., Turkonyi E., Bramlage P., Zannad F. (2011). Biomarkers: optimizing treatment guidance in heart failure. *Clinical Research in Cardiology* 100, 973-981.
- Braunwald E. (2008). Biomarkers in heart failure. *The New England Journal of Medicine* 358, 2148-2159.
- Buckley M.G., Marcus N.J., Yacoub M.H. (1999). Cardiac peptide stability, aprotinin and room temperature: importance for assessing cardiac function in clinical practice. *Clinical Science* 97, 689-695.
- Chiang F. T., Tseng C. D., Hsu K. L., Lo H. M., Tseng Y. Z., Hsieh P. S., Wu T. L. (1994). Circadian variations of atrial-natriuretic-peptide in normal people and its relationship to arterial blood-pressure, plasma-renin activity and aldosterone level. *International Journal of Cardiology* 46, 229-233.
- Christenson R. H., Apple F. S., Morgan D. L., Alonsozana G. L., Mascotti K., Olson M., McCormack R. T., Wians F. H., Keffer J. H., Duh S. H. (1998). Cardiac troponin I measurement with the ACCESS (R) immunoassay system: analytical and clinical performance characteristics. *Clinical Chemistry* 44, 52-60.
- Decloedt A., Verheyen T., De Clercq D., Sys S., Vercauteren G., Ducatelle R., Delahaut P., Van Loon G. (2012). Acute and long-term cardiomyopathy and delayed neurotoxicity after accidental lasalocid poisoning in horses. *Journal of Veterinary Internal Medicine* 26, 1005-1011.
- Divers T. J., Kraus M. S., Jesty S. A., Miller A. D., Mohammed H. O., Gelzer A. R. M., Mitchell L. M., Soderholm L. V., Ducharme N. G. (2009). Clinical findings and serum cardiac troponin I concentrations in horses after intragastric administration of sodium monensin. *Journal of Veterinary Diagnostic Investigation* 21, 338-343.
- Ettinger S. J., Farace G., Forney S. D., Frye M., Beardow A. (2012). Evaluation of plasma N-terminal pro-B-type natriuretic peptide concentrations in dogs with and without cardiac disease. *Javma-Journal of the American Veterinary Medical Association* 240, 171-180.
- Evans M. J., Livesey J. H., Ellis M. J., Yandle T. G. (2001). Effect of anticoagulants and storage temperatures on stability of plasma and serum hormones. *Clinical Biochemistry* 34, 107-112.
- Felker G. M., Petersen J. W., Mark D. B. (2006). Natriuretic peptides in the diagnosis and management of heart failure. *Canadian Medical Association journal* 175, 611-617.
- Fox P. R., Oyama M. A., Reynolds C., Rush J. E., Defranco T. C., Keene B.W., Atkins C. E., Macdonald K. A., Schober K.E., Bonagura J. D., Stepien R. L., Kellihan H. B., Nguyenba T.P., Lehmkuhl L.B., Lefbom B.K., Moise N.S., Hogan D.F. (2009). Utility of plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) to distinguish between congestive heart failure and non-cardiac causes of acute dyspnea in cats. *Journal of Veterinary Cardiology* 11, 51-61.
- Gehlen H., Rohn K., Deegen E., Stadler P. (2006). Labor-diagnostische Untersuchungen bei Pferden mit Herzerkrankungen: Wertigkeit verschiedener kardialer Marker. *Pferdeheilkunde* 22, 532-541.
- Gehlen H., Sundermann T., Rohn K., Stadler P. (2008). Aldosterone plasma concentration in horses with heart valve insufficiencies. *Research in Veterinary Science* 85, 340-344.
- Gehlen H., Sundermann T., Rohn K., Stadler P. R. (2007). Plasma atrial natriuretic peptide concentration in warm-blood horses with heart valve regurgitations. *Journal of Veterinary Cardiology* 9, 99-101.
- Ghosh N., Haddad H. (2011). Atrial natriuretic peptides in heart failure: pathophysiological significance, diagnostic and prognostic value. *Canadian Journal of Physiology and Pharmacology* 89, 587-591.
- Goetze J. P., Kastrup J., Rehfeld J. F. (2003). The paradox of increased natriuretic hormones in congestive heart failure patients: Does the endocrine heart also fail in heart failure? *European Heart Journal* 24, 1471-1472.
- Goetze J.P. (2004a). Biochemistry of pro-B-type natriuretic peptide derived-peptides: the endocrine heart revisited. *Clinical Chemistry* 1503-1510.
- Goetze J.P. (2004b). ProBNP-derived peptides in cardiac disease. *Scandinavian Journal of Clinical and Laboratory Investigation* 64, 497-509.
- Greco D.S., Biller B., Van Liew C.H. (2003). Measurement of plasma atrial natriuretic peptide as an indicator of prognosis in dogs with cardiac disease. *Canadian veterinary journal* 44, 293-297.
- Guthrie G. P., Cecil S. G., Darden E. D., Kotchen T. A. (1982). Dynamics of renin and aldosterone in the Thoroughbred horse. *General and Comparative Endocrinology* 48, 296-299.
- Haggstrom J., Hansson K., Kvart C., Karlberg B. E., Vuolteenaho O., Olsson K. (1997). Effects of naturally acquired decompensated mitral valve regurgitation on the renin-angiotensin-aldosterone system and atrial natriuretic peptide concentration in dogs. *American Journal of Veterinary Research* 58, 77-82.

- Hall C. (2005). NT-ProBNP: The mechanism behind the marker. *Journal of Cardiac Failure* 11, 81-83.
- Harris P. A. (1993). Preliminary investigations into factors that affect plasma-aldosterone concentrations in horses. *Research in Veterinary Science* 54, 319-328.
- Hayek S., Nemer M. (2010). Cardiac natriuretic peptides: from basic discovery to clinical practice *Cardiovascular Therapeutics* 29, 362-376.
- Herndon W. E., Rishniw M., Schroppe D., Sammarco C. D., Boddy K. N., Sleeper M. M. (2008). Assessment of plasma cardiac troponin I concentration as a means to differentiate cardiac and noncardiac causes of dyspnea in cats. *Journal of the American Veterinary Medical Association* 233, 1261-1264.
- Hori Y., Ohshima N., Chikazawa S., Kanai K., Hoshi F., Itoh N., Higuchi S. (2012). Myocardial injury-related changes in plasma NT-proBNP and ANP concentrations in a canine model of ischemic myocardial injury. *The Veterinary Journal* 191, 46-51.
- Hori Y., Yamano S., Iwanaga K., Kano T., Tanabe M., Uechi M., Kanai K., Nakao R., Hoshi F., Higuchi S. (2008). Evaluation of plasma C-terminal atrial natriuretic peptide in healthy cats and cats with heart disease. *Journal of Veterinary Internal Medicine* 22, 135-139.
- Hori Y., Yamano S., Kanai K., Hoshi F., Itoh N., Higuchi S. (2011). Clinical implications of measurement of plasma atrial natriuretic peptide concentration in dogs with spontaneous heart disease. *Javma-Journal of the American Veterinary Medical Association* 239, 1077-1083.
- Hsu A., Kittleson M.D., Paling A. (2009). Investigation into the use of plasma NT-proBNP concentration to screen for feline hypertrophic cardiomyopathy. *Journal of Veterinary Cardiology* 11, 63-70.
- Jaffe A. S., Vasile V. C., Milone M., Saenger A. K., Olson K. N., Apple F. S. (2011). Diseased skeletal muscle: a non-cardiac source of increased circulating concentrations of cardiac troponin T. *Journal of the American College of Cardiology* 58, 1819-1824.
- Jansson A., Dahlborn K. (1999). Effects of feeding frequency and voluntary salt intake on fluid and electrolyte regulation in athletic horses. *Journal of Applied Physiology* 86, 1610-1616.
- Kemperman H., Van Den Berg M., Kirkels H., De Jonge N. (2004). B-type natriuretic peptide (BNP) and N-terminal proBNP in patients with end-stage heart failure supported by a left ventricular assist device. *Clinical Chemistry* 50, 1670-1672.
- Kimura K., Yamaguchi Y., Horii M., Kawata H., Yamamoto H., Uemura S., Saito Y. (2007). ANP is cleared much faster than BNP in patients with congestive heart failure. *European Journal of Clinical Pharmacology* 63, 699-702.
- Kokkonen U.-M. (2002). Plasma atrial natriuretic peptides in the horse and goat with special reference to exercising horses. *PhD thesis*. Faculty of Veterinary Medicine of the University of Helsinki, Helsinki.
- Kokkonen U.-M., Pösö A.R., Hyppä S., Huttunen P., Lepäluoto J. (2002). Exercise-induced changes in atrial peptides in relation to neuroendocrine responses and fluid balance in the horse. *Journal of Veterinary Medicine* 49, 144-150.
- Kraus M. S., Jesty S. A., Gelzer A. R., Ducharme N. G., Mohammed H. O., Mitchell L. M., Soderholm L. V., Divers T. J. (2010). Measurement of plasma cardiac troponin I concentration by use of a point-of-care analyzer in clinically normal horses and horses with experimentally induced cardiac disease. *American Journal of Veterinary Research* 71, 55-59.
- Kraus M. S., Kaufer B. B., Damiani A., Osterrieder N., Rishniw M., Schwark W., Gelzer A. R., Divers T. J. (2011). Pharmacokinetics of intravenously administered equine cardiac troponin I in healthy ponies. *Journal of Veterinary Internal Medicine* 25, 669-669.
- Linklater A. K. J., Lichtenberger M. K., Thamm D. H., Tillery L., Kirby R. (2007). Serum concentrations of cardiac troponin I and cardiac troponin T in dogs with class IV congestive heart failure due to mitral valve disease. *Journal of Veterinary Emergency and Critical Care* 17, 243-249.
- Maisel A. S., Krishnaswamy P., Nowak R. M., Mccord J., Hollander J. E., Duc P., Omland T., Storrow A. B., Abraham W. T., Wu A. H. B., Clopton P. G., Westheim A., Knudsen C. W., Perez A., Kazanegra R., Herrmann H. C., Mccullough P. A. (2002). Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. *New England Journal of Medicine* 347, 161-167.
- Maynard S. J., Menown I. B. A., Adgey A. A. J. (2000). Troponin T or troponin I as cardiac markers in ischaemic heart disease. *Heart* 83, 371-373.
- Mcgurrian M. K. J., Physick-Sheard P. W., Kenney D. G. (2008). Transvenous electrical cardioversion of equine atrial fibrillation: patient factors and clinical results in 72 treatment episodes. *Journal of Veterinary Internal Medicine* 22, 609-615.
- Mifune H., Richter R., Forssmann W. G. (1995). Detection of immunoreactive atrial and brain natriuretic peptides in the equine atrium. *Anatomy and Embryology* 192, 117-121.
- Muñoz A., Riber C., Trigo P., Castejón F. M. (2010). Clinical applications of the renin-angiotensin-aldosterone-vasopressin axis in the horse and future directions for research. *Journal of Equine Veterinary Science* 30, 607-316.
- Nelesen R.A., Dimsdale J.E., Ziegler M.G. (1992). Plasma atrial natriuretic peptide is unstable under most storage conditions. *Circulation* 86, 463-466.
- Nostell K., Hågström J. (2008). Resting concentrations of cardiac troponin I in fit horses and effect of racing. *Journal of Veterinary Cardiology* 10, 105-109.
- O'Brien P.J., Dameron G.W., Beck M.L., Brandt M. (1998). Differential reactivity of cardiac and skeletal muscle from various species in two generations of cardiac troponin-T immunoassays. *Research in Veterinary Science* 65, 135-137.
- Oyama M. A., Sisson D. D. (2004). Cardiac troponin-I concentration in dogs with cardiac disease. *Journal of Veterinary Internal Medicine* 18, 831-839.
- Parnacek M. S., Solaro R. J. (2004). Biology of the troponin complex in cardiac myocytes. *Progress in Cardiovascular Diseases* 47, 159-176.
- Payne E. E., Roberts B. K., Schroeder N., Burk R. L., Schermerhorn T. (2011). Assessment of a point-of-care cardiac troponin I test to differentiate cardiac from noncardiac causes of respiratory distress in dogs. *Journal of Veterinary Emergency and Critical Care* 21, 217-225.
- Phillips W., Giguère S., Franklin R.P., Hernandez J., Adin D., Peloso J. (2003). Cardiac troponin I in pastured and race-training Thoroughbred horses. *Journal of Veterinary Internal Medicine* 17, 597-599.
- Richter R., Magert H. J., Mifune H., Schulz-Knappe P., Forssmann W. G. (1998). Equine cardiodilatin atrial natriuretic peptide- Primary structure and immunohistochemical localization in auricular cardiocytes. *Acta Anatomica* 162, 185-193.

- Rogers R. K., Stehlik J., Stoddard G.J., Greene T., Collins S. P., Peacock W. F., Maisel A. D., Clopton P., Michaels A. D. (2009). Adjusting for clinical covariates improves the ability of B-type natriuretic peptide to distinguish cardiac from non-cardiac dyspnoea: a sub-study of HEARD-IT. *European Journal of Heart Failure* 11, 1043-1049.
- Saito Y. (2010). Roles of atrial natriuretic peptide and its therapeutic use. *Journal of Cardiology* 56, 262-270.
- Sandersen C., Remy B., Olejnik D., Amory H. (2008). Intérêt de la troponine sérique en tant que marqueur d'une myocarde en médecine vétérinaire. *Annales de Médecine Vétérinaire* 152, 180-190.
- Schwarzwald C.C., Hardy J., Buccellato M. (2003). High cardiac troponin I serum concentration in a horse with multifocal ventricular tachycardia and myocardial necrosis. *Journal of Veterinary Internal Medicine* 17, 364-368.
- Takei Y., Inoue K., Trajanovska S., Donald J. A. (2011). B-type natriuretic peptide (BNP), not ANP, is the principal cardiac natriuretic peptide in vertebrates as revealed by comparative studies. *General and Comparative Endocrinology* 171, 258-266.
- Tate R. T., Panthegini M. (2008). Measurement of cardiac troponins revisited. *Biochimica Clinica* 32, 535-546.
- Thibault G., Murthy K. K., Gutkowska J., Seidah N. G., Lazure C., Chrétien M., Catin M. (1987). NH₂-Terminal fragment of rat pro-atrial natriuretic factor in the circulation: identification, radioimmunoassay and half-life. *Peptides* 9, 47-53.
- Trachsel D.S., Grenacher B., Weishaupt M.A., Schwarzwald C.C. (2011). Plasma atrial natriuretic peptide concentrations in horses with heart disease: A pilot study. *The Veterinary Journal*, 1-5.
- Tsai S. H., Lin Y. Y., Chu S. J., Hsu C. W., Cheng S. M. (2010). Interpretation and use of natriuretic peptides in non-congestive heart failure settings. *Yonsei Medical Journal* 51, 151-163.
- Verheyen T., Decloedt A., De Clercq D., Van Loon G. (2012). Cardiac changes in horses with atypical myopathy. *Journal of Veterinary Internal Medicine* 26, 1019-1026.
- Weber K. T. (2001). Aldosterone in congestive heart failure. *The New England Journal of Medicine* 345, 1689-1697.
- Wells S. M., Sleeper M. (2008). Cardiac troponins. *Journal of Veterinary Emergency and Critical Care* 18, 235-245.
- Woodard G. E., Rosado J. A. (2007). Recent advances in natriuretic peptide research. *Journal of Cellular and Molecular Medicine* 11, 1263-1271.
- Zolty R., Bauer C., Allen P., Garrity M., Vittorio T.J. (2008). Atrial natriuretic peptide stability. *Clinical Biochemistry* 41, 1255-1258.