Cardiac biomarkers for early detection of heart diseases in small animals

Changbaig Hyun, DVM, MVetClinStud, PhD
Section of Small Animal Internal Medicine, School of Veterinary Medicine, Chuncheon, Korea

ABSTRACT

Background: Heart failure can develop secondary to various heart diseases (e.g. mitral valvular insufficiency, congenital heart defects, heart worm infection). The clinical signs of heart failure can be confused with those of other conditions, such as respiratory disease. Therefore, specific, sensitive, rapid and inexpensive blood tests for heart failure are desirable. Cardiac troponins, natriuretic peptides and cytokines have been more recently used as
indicators of heart disease in humans and animals. These peptides are sensitive to changes in vasoconstriction and dilation within the heart and are used for the diagnosis and prognosis of heart failure.

Methods: Previously developed and newly developed cardiac biomarkers will be discussed for understating clinical implications and diagnostic values in heart diseases in small animals

INTRODUCTION

Congestive heart failure (CHF) is a condition that can result from any structural or functional cardiac disorder that impairs the ability of the heart to fill with or pump a sufficient amount of blood through the body. The most common causes of heart failure are valvular heart disease, congenital heart disease,primary cardiomyopathies and heart worm infections in dogs and cats. Probably chronic mitral valvular insufficiency (CMVI) is the most common cause of CHF in Korea, because small breed dogs are over-presented. CMVIis caused by a myxomatous degenerative change in the subendocardial mitral valve leaflets and chordae tendineae in middle-aged to elderly dogs. CMVI accounts for about 75% of the cardiovascular disease cases seen in dogs where it is most prevalent in smaller breeds such as the Shih Tzus, Malteses, Yorkshire Terriers and Toy Poodles [26, 37]. Although the etiology of CMVI is not fully understood, familial CMVI has been well documented in Cavalier King Charles Spaniels [9, 31]. In CMVI, the regurgitant flow from the left

ventricle (LV) to the left atrium (LA) due to mitral valvular insufficiency can cause marked LA and LV dilation (eccentric hypertrophy) that leads to left-sided congestive heart failure often accompanied with life-threatening pulmonary edema [30].

Recent advances in veterinary diagnostic imaging technology have enabled us to detect CHFearlier and more accurately. However, when only in the hands of a specialist or someone with extensive training in cardiology does diagnostic imaging provide an accurate picture of a state of a disease or its prognosis. In this sense, considerable research has been conducted into the use of cardiac biomarkers to allow an easy but reliable method to evaluate cardiac diseases such as CMVI for more accurate staging of CMVI in dogs. Since then, several potential cardiac biomarkers have been identified and tested for use in the diagnosis of heart failure in dogs such as, atrial natriuretic peptide (ANP) [5, 25, 57], brain natriuretic peptide (BNP) [21, 43, 57], N-terminal proANP fragments (NT-pro ANP) [12, 26, 57], and cardiac troponins (cTn) [57, 64]. In human medicine research is not only targeted on circulating biochemical markers but also on expression patterns of particular genes that can precisely reflect the diverse and complicated hemodynamic changes of different cardiovascular abnormalities [38, 41, 62]. Regulations of potential genes are being assessed via real-time RT-PCR or microarray analysis that is being widely investigated in cardiology. Furthermore, current studies are being focused on gene expression levels in the blood instead of previously employed heart or tissue samples due to its noninvasiveness and accessibility.

CARDIAC BIOMARKERS : CLINICAL IMPLICATIONS AND DIAGNOSTIC VALUE

1. Muscle enzymes

Serum levels of the muscle enzymes AST (aspartate aminotransferase), LD (lactate dehydrogenase) and CK(creatine kinase) were regarded as diagnostic markers until isoenzymes of LD and CK wereidentified. Cardiac muscle-specific CK-MB isoenzyme and LD1 and 2 isoenzymes become recognised as markers of acute significant myocardial infarction (AMI) in humans. However, it was later recognised that these assays have low specificity and sensitivity in the presence of skeletal muscle injury, in both humans [1] and animals [35].In large animals such as equine athletes and large breed dogs this could become a serious confounding factor. Chronic renal disease was also found to affect serum levels of CK-MB [35]. In dogs these problems are compounded by low levels of CK-MB in myocardium compared to humans, constituting 4%?15% and 40% of total CK, respectively. In dogs this isoenzyme is found in skeletal muscle, lung, spleen and intestine [7].

2. Troponins

The criteria for the diagnosis of AMI in humans have recently been redefined [3] and troponins designated as the preferred biomarkers for cardiac injury. The muscle troponin?tropomyosin complex contains three troponins, C, T and I [17, 34]. This complex was first described in 1965 by Ebashi (1980) [23].

Troponin C has two isoforms in cardiac and skeletal muscle and contains a calcium–binding site which regulates muscle contraction, as well as magnesium–binding sites, and is highly conserved [34]. Troponin T, the largest troponin, does not bind calcium but changes in its variable region alter conformation and interactions within the troponin? tropomyosin?actin complex in thin filaments of the sarcomere. Four skeletal muscle and four cardiac isoforms have been identified. Troponin I binds to actin, tropomyosin, TnT and TnC, and contains multiple phosphorylation sites. Among these are two serine residues which, when phosphorylated, induce a conformation change in the molecule that regulates calcium sensitivity andactinomyosin ATPase activity within the complex. There are two cardiac and skeletal muscle isoforms, but no fetal forms have been identified in cardiac muscle.

Circulating levels of both cTnT and cTnI are extremely low or undetectable by current assay methods in healthy mammals. With cardiac myocyte injury both troponins are released into the circulation, and halflives of 120 min have been reported for cTnT in humans [17] and 2 h for cTnT and cTnI in dogs [61]. Both cTnT and cTnI are released biphasicallypost myocardial ischaemia and necrosis, and remain elevated from 5 to 20 days, depending on the severity of injury and reperfusion [72]. These molecules can be modified by both proteolysis and degradation of both N and C terminal regions, leading to a mixture of circulating intact and fragment molecules [39]. Circulating levels of both cTnT and cTnI have been correlated to the extent of myocardial damage in humans [17, 55] and dogs [52, 53].

Troponins are phylogenetically highly conserved proteins with 〉95% homology between mammals, and so assays developed for human use can often be validated and adapted for use in animals. Immunoassays of various configurations have been developed for both cTnT and cTnI in human blood. Extensive evaluation studies of these different assays indicate that they are both cardiospecific and reliable [4, 17, 70]. Both cTnT and cTnI are stable in serum, EDTA and heparin plasma for 24 h at room temperature, 1 week at 4 C, and 〉1 month frozen, and are not affected by freeze?thawing.

Both cTnT and cTnI assays have been used in animals in experimental and clinical settings. Cardiac troponin T was found to be cardiospecific and a sensitive biomarker for cardiac injury in laboratory animals, including dogs and mice [51, 74]. With second generation assays cTnT was found in skeletal muscle in dogs at 100th of the concentration found in the heart. However,

concern was expressed about the effect of this on the interpretation of cTnT levels in large animals [52]. Biopsies of heart and skeletal muscle in dogs and horses demonstrated that levels of cTnI were 1000 times greater in heart than in skeletal muscle.

The quantities of cTnI per gram of tissue were also higher in cardiac tissue of large animals (dogs, horses, cows, sheep, pigs) than in small ones (rats, mice, marmosets) [53]. Serum cTnI levels were found to be more markedly increased and in more dogs with blunt chest trauma than cTnT levels [61]. In canine babesiosis cTnT was also reported to be less sensitive than cTnI in detecting myocardial damage [42]. Serum levels of both cTnT and cTnI were increased in dogs with gastric dilation?volvulus [60]. Both cTnT and cTnI were found to be sensitive markers of cardiotoxicity in experimental [27, 51, 74] and clinical [21] doxorubicin therapy. A human assay for cTnI was validated in healthy dogs and cats, providing reference ranges similar to those reported for humans [63]. Similar values were also found in normal horses, but increased values were found in a horse with 'jet lesions' and horses with histologically confirmed myonecrosis [18]. Interestingly, in dogs with dilated cardiomyopathy, levels of cTnT in cardiac myofibres were 30% lower than in normal myocardium [54]. Recent studies in cats with hypertrophic cardiomyopathy (HCM) demonstrated a correlation between increasing levels of serum cTnI, severity of clinical signs and echocardiographic changes [15]. A correlation between increased serum levels of cTnI and CHF in cats with HCM was also recently reported [28].

3. Natriuretic peptides

The natriuretic, diuretic and vasorelaxant activities of this family of small peptides were first demonstrated in rats administered extracts of cardiac tissue [20]. Atrial natriuretic peptide (ANP) was the first to be isolated [33], followed by brain natriuretic peptide (BNP), first isolated from porcine brain [66] and C-type natriuretic peptide (CNP).

Atrial natriuretic peptide (ANP) is produced in health by the cells of the atria, primarily in response to increased atrial tension and stretch followed by increased levels of endothelins and angiotensin II. Prepro-ANP has been reported in fetal ventricle myocytes but not adult myocytes; however, it is reported to be upregulated with hypertrophy and damage. In chronic heart failure (CHF) large amounts can be produced by ventricle cells and released into the circulation [36]. The plasma half-life of ANP is around 3 min, but that of pro-ANP is approximately 10times longer, leading to much higher circulating levels of the latter, particularly following injury.

Brain natriuretic peptide (BNP) was initially isolated from brain it is more abundant in cardiac ventricles [69] and is produced in response to similar stimuli to those of ANP. Prepro-BNP, a 131aa peptide is reduced to a 108aa pro-BNP. This in turn is broken into an active BNP form of 32aa, and

an N-terminal pro-BNP of 76aa. This is believed to occur within or on the surface of the cardiac myocyte [44]. Both enter the circulation, with the concentration of pro- BNP being approximately 10 times that of BNP. The plasma half-life is reported to be similar to that of ANP. Little is known of the biological function or metabolism of circulating pro-BNP. With CHF and ventricular hypertrophy, circulating levels are markedly increased [40].

Measurement of natriuretic peptides is considered to provide a better index of disease than catecholamines, renin, angiotensin, aldosterone or endothelin. However, circulating levels of natriuretic peptides, particularly ANP, can be increased by any increase in atrial tension, central blood volume or oedema, which may be secondary to renal or hepatic disease [44]. Levels of plasma pro-ANP (NT-pro ANP) 31?67 were elevated in athletes compared to persons with a more sedentary lifestyle [48].

N-terminal (1?98) pro-ANP was found to have 97% sensitivity and 92% specificity in symptomatic CHF [49]. It was also found to be of greater prognostic value than ANP post AMI, in dilated cardiomyopathy and in valvular disease [19, 73]. BNP was found to have 97% sensitivity and 84% specificity in a similar study. However, BNP and pro-BNP have become the tests of choice for predicting recovery in various types of human heart disease [36, 44, 46]. Plasma levels can be reduced by furosemide therapy without affecting echocardiographic findings, and increased by b-blockers [40]. They have also been used to monitor doxorubicin cardiotoxicity in lymphoma therapy in humans [50]. The BNP assay had a sensitivity of 93% and specificity of 79%, whereas for the pro-BNP assay the values were 90% and 66%, respectively [24], again confirming the value of these tests in an emergency setting to accurately predict in particular the severity of change in LVEF (left ventricular ejection fraction). Rapid BNP assays were demonstrated in a large human study (1586 persons with acute dyspnoea) to be of value in establishing or excluding a diagnosis of CHF [45].

Early work with dogs as models demonstrated that, as in humans, ANP inhibited the renin-angiotensin-aldosterone pathway, and that this was marked in CHF [71]. In later studies it was found that in dogs with CHF, large increases in ANP appeared to induce rapid progress of CHF, possibly secondary to decreased renal responsiveness in the face of increased circulating levels [65]. Atrial natriuretic peptide was localised to the atria in normal dogs by immunohistochemistry, whereas in dilated cardiomyopathy it was reduced in atria and increased in ventricle myocytes [16]. In studies of dogs with clinical mitral regurgitation using RIA for human pro-ANP and ANP and porcine BNP in solid-phase extracted plasma, levels of pro-ANP and ANP were increased three to seven times above controls, whereas BNP values were increased twofold. Using ROC (receiver operator curves) it was established that levels of circulating pro-ANP were best for detecting

decompensating HF in these dogs [8]. Studies with a newer ELISA against synthetic canine pro-ANP 31-67 fragments in unextracted EDTA plasma from healthy dogs, and those with HF analysed by ROC using a cut off level of 1750 fm/l, demonstrated a sensitivity of 83.9% and a specificity of 97.5% for detecting HF in dogs [12]. A similar ELISA for human pro-ANP 1?98 was validated for use in cats. However, in cats with increased serum creatinine and chronic renal disease levels were elevated [67]. Because of the variable homology between species for natriuretic peptides, recent work has focused on cloning species-specific genes and defining the nucleotide and peptide aa. sequences. Recently the gene for feline ANP was isolated and the aa sequences for the peptides deduced [11]. The N-terminal pro-ANP 1-98 was found to have 94% homology with both human and horse, the active ANP 1-30 being identical in cat, horse, cow and sheep, and differing from human in the two terminal arginine residues. The gene for canine BNP has also been cloned [2, 6]. High homology was reported for BNP in dog, cow, pig and sheep. With the identification of aa sequences in pro-ANP and pro-BNP fragments the development of canine and feline specific assays is anticipated.

4. Cytokines

Cardiotrophins are relatively recently identified peptides in the IL-6 family of cytokines. Cardiotrophin-1 (CT-1) is a 201aa peptide produced by both cardiac myocytes and fibroblasts, and is activated and modulated through its receptor component gp130/LIF receptor (leukaemia inhibitory factor) through a coupled signalling pathway JAK/STAT (Janus kinase signal transduction and activators of transcription). Through these pathways CT-1 induces cardiac myocyte hypertrophy [32, 46, 75]. Mechanical stretch of myocytes apparently induces increased production of CT-1 [14]. Expression is also increased in the presence of hypoxia and reperfusion to protect myocytes from ischaemia, reperfusion injury and apoptosis. It also activates myocyte hypertrophy in the presence of volume overload, leading to increased myocyte length by the addition of sarcomere units causing ventricular dilation [68].

Increased plasma levels have been reported in human chronic and acute heart failure [75]. Noncompetitive assays are available [47, 68]. By the non-competitive assay using antibodies against the midsection aa 105?102 and C-terminal aa 186-199, plasma levels in humans in heart failure were three times those found in healthy individuals [47]. It has been suggested that levels of CT-1 are increased in plasma in animal models and human HF before pathological hypertrophy changes are seen [15]. Raised plasma CT-1 levels have been reported 7 days post AMI, and it has been suggested that it may be an earlier marker of myocyte damage than BNP, which is frequently used in human medicine [68].

Osteopontin (OPN) also referred to as cytokine Eta-1, plays an important role in functional and pathologic cardiac remodeling. It is a nonstructural secreted matrix protein containing the arginine-glycine-aspartic acid-serine cell-binding sequence found in many extracellular matrix proteins. In healthy animals, OPN is found mainly in bone and epithelial tissues, but, in thepathologic circumstances, it is produced in abundant quantities by endothelial cells, smooth muscle cells, fibroblasts, and cardiomyocytes. Cardiomyocyte OPN expression increases, together with integrin expression, in response to pressure overload mechanical stress, these alterations are accompanied by increased activity of numerous signaling pathways that lead to the induction of concentric myocardial hypertrophy. Myocardial sources of angiotensin (AT) II and TGF-a1 also induce the expression of OPN in concert with the development and progression of myocardial hypertrophy as demonstrated in a variety of heart failure and hypertension models. Osteopontin expression also increases in cardiac fibroblasts and endothelial cells in response to-activation of the RAAS. Marked upregulation of ATI receptors by aldosterone makes it difficult to determine if OPN production is induced directly by aldosterone or if its activation is exclusively or primarily accomplished via ATII.

Plasma levels of OPN are elevated in human patients with heart failure due to ischemic or dilated cardiomyopathy; OPN levels correlate with NYHA class and appear to predict mortality independent of NT-pro-BNP levels. Preliminary studies conducted in our laboratory showed that OPN expression was modestly increased in the hearts of dogs with dilated cardiomyopathy and were more dramatically increased in the hearts of cats with hypertrophic or restrictive cardiomyopathy. However, increased OPN expression (via immunohistochemistry) was not detected in the hearts of dogs with end-stage mitral regurgitation either in cardiac myocytes or in interstitial fibroblasts.

5. New markers

The sodium-calcium exchanger (NCX-1) is an important gene involved in cardiac excitation-contraction coupling, which is correlated to the calcium (Ca2+) levels in cardiac myocytes[10, 29, 56, 58]. The up-regulation of NCX-1 expression has been observed in mice with cardiac hypertrophy and heart failure, where the magnitude of NCX-1 expression increased in proportion to the cardiac stress and heart failure observed [59]. As a result of these studies, we recently investigated the level of NCX-1 gene expression in dogs with different stages of CMVI using real-time reverse transcription polymerase chain reaction (RT-PCR). The fold differences in the levels of mRNA expression compared to controls were 1.39 ± 0.88 in group I, 1.32 ± 0.65 in group II, 4.86 ± 1.25 in group III, and 5.96 ±1.69 in group IV. The expression of NCX-1 was significantly

increased in groups III to IV ($P < .05$), while expression levels in groups I to II were not significant compared to healthy controls. The level of NCX-1 expression increased significantly in groups of dogs having moderate to severe CMVI. The NCX-1 can be an alternative cardiac biomarker for compensating limitations from cardiac biomarkers previously studied in veterinary fields.

CONCLUSION

Data from human patients and emerging data from canine populations suggest that cardiac biomarkers are useful in discriminating patients with clinical signs from those without clinical signs. Elevated concentrations may be indicative of more advanced stages of cardiac remodeling. Concentrations of certain natiriuretic peptides (e.g. NTproBNP)in groups of dogs destined to develop clinical signs in the near future appear higher than in those that do not develop signs. Finally higher concentrations in our population appear predictive of a greater likelihood of death due to cardiac causes. However no single biomarker may correctly predict the state of heart failure in dogs and cats. Furthermore each biomarker has limitations for correct diagnosis and prognosis for heart failure. Therefore future studies should direct to develop new cardiac biomarkers which can compensate the limitations from pre-existed cardiac biomarkers and to develop multiple cardiac biomarker analysis for more accurate diagnosis and prognosis.

REFERENCE

1. Adams JE 3rd, Schechtman KB, Landt Y, Ladenson JH, Jaffe AS. Comparable detection of acute myocardial infarction by creatine kinase MB isoenzyme and cardiac troponin I. Clin Chem, 1994; 40:1291?1295.

2. Aitken GD, Raizis AM, Yandle TG, George PM, Espiner EA, Cameron VA. The characterization of ovine genes for atrial, brain and C-type natriuretic peptides. Dom Anim Endocrinol, 1999; 16:115-121.

3. Alpert J, Thygesen K. Myocardial infarction redefined: A consensus document of the joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol, 2000; 36:959?969.

4. Armstrong GP, Barker AN, Patel H, Hart HH. Reference intervals for troponin I on the ACS: Cenataurassay: A recommendation based on the recent redefinition of myocardial infarction. Clin Chem, 2002; 48:198-199.

5. Asano K, Masuda K, Okumura M, Kadosawa T, Fujinaga T. Plasma atrial and brain natriuretic peptide levels in dogs with congestive heart failure. J Vet Med Sci, 1999; 61:523-529.

6. Asano K, Murakami M, Endo D, Kimura T, Fujinaga T. (1999a) Complementary DNA

cloning, tissue distribution, and synthesis of canine brain natriuretic peptide. Am J Vet Res, 1999a; 60:860-864.

7. Atkas A, Auguste D, Lefebrve E. Creatine kinase in the dog: A review. Vet Res Comm, 1993; 17:353-369.

8. Axelsson M, Eksell P, Ron?us B, Brostr?m H, H?ggstr?m J, Carlsten J. Relationship between different natriuretic peptides and severity of naturally acquired mitral regurgitation in dogs with chronic myxomatous valve disease. J Vet Cardiol, 2000; 2:7-16.

9. Beardow AW, Buchanan JW. Chronic mitral valve disease in cavalier King Charles spaniels: 95 cases (1987-1991). J Am Vet Med Assoc, 1993; 203:1023-1029.

10. Bers DM. Cardiac excitation-contraction coupling. Nature, 2002; 415:198-205.

11. Biondo AW, Liu ZL, Wiedmeyer CE, de Morais HS, Sisson DD, Solter PE. Genomic sequence and cardiac expression of atrial natriuretic peptide in cats. Am J Vet Res, 2002; 63:236-240.

12. Boswood A, Attree S, Page K. Clinical validation of a pro- ANP 31?67 fragment ELISA in the diagnosis of naturally occurring canine heart failure. J Sm Anim Pract, 2001; 42:365.

13. Boswood A, Attree S, Page K. Clinical validation of a proANP 31-67 fragment ELISA in the diagnosis of heart failure in the dog. J Small Anim Pract, 2003; 44:104-108.

14. Bristow MR, Long CS. Cardiotrophin-1 in heart failure: Editorial. Circulation, 2002; 106:1430-1432.

15. Cannata J, Connolly D, Archer J. Hypertrophic cardiomyopathy in cats: comparison of echocardiographic changes and cardiac troponin I. J Vet Clin Pathol, 2002; 31:203.

16. Colbatzky F, Vollmar A, M?nch U, Hermanns W. Synthesis and distribution of atrial natriuretic peptide (ANP) in hearts from normal dogs and those with cardiac abnormalities. J Comp Pathol, 1993; 108:149-163.

17. Collinson PO, Boa FG, Gaze DC. Measurement of cardiac troponins. Ann Clin Biochem, 2001; 38:423-449.

18. Cornelisse CJ, Schott HC 2nd, Olivier NB, Mullaney TP, Koller A, Wilson DV, Derksen FJ. Concentrations of troponin I in a horse with a ruptured aortic regurgitation jet lesion and ventricular tachycardia. J Am Vet Med Assoc, 2000; 217:231-235.

19. Cowie MR, Struthers AD, Wood DA, Coats AJ, Thompson SG, Poole-Wilson PA, Sutton GC. Value of natriuretic peptides in assessment of patients with possible new heart failure in primary care. Lancet, 1997; 350:1347-1351.

20. de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extracts in rats. Life Sci, 1981; 28:89-94.

21. DeFrancesco TC, Rush JE, Rozanski EA, Hansen BD, Keene BW, Moore DT, Atkins CE. Prospective clinical evaluation of an ELISA B-type natriuretic peptide assay in the diagnosis of congestive heart failure in dogs presenting with cough or dyspnea. J Vet Intern Med, 2007; 21:243-250.

22. DeFrancesco TC, Rush JE, Rozanski EA, Hansen BD, Keene BW, Moore DT, Atkins CE. Prospective clinical evaluation of serum cardiac troponin T in dogs admitted to a Veterinary Teaching Hospital. J Vet Intern Med, 2002; 16:553-557.

23. Ebashi S. The Croonian lecture, 1979: Regulation of muscle contraction. Proc Roy Soc Lond B Biol Sci, 1980; 207:259-286.

24. Fischer Y, Filzmaier K, Stiegler H, Graf J, Fuhs S, Franke A, Janssens U,Gressner AM. Evaluation of a new rapid bedside test for quantitative determination of B-type natriuretic peptide. Clin Chem, 2001; 47:591-594.

25. Greco DS, Biller B, Van Liew CH. Measurement of plasma atrial natriuretic peptide as an indicator of prognosis in dogs with cardiac disease. Can Vet J, 2003; 44:293-297.

26. Haggstrom J, Kvart C, Pedersen HD. Aquired Valvular Heart Disease. In: Ettinger SJ and Feldman EC (ed.), Textbook of Veterinary Internal Medicine. Elsevier, St. Louis, Unite State. 2005: 1022-1039.

27. Herman EH, Zhang J, Lipshultz SE, Rifai N, Chadwick D, Takeda K, Yu ZX, Ferrans VJ. Correlation between serum levels of cardiac troponin-T and severity of chronic cardiomyopathy induced by doxorubicin. J Clin Oncol, 1999; 17:2337-2345.

28. Herndon WE, Kittleson MD, Sanderson K, Drobatz KJ, Clifford CA, Gelzer A, Summerfield NJ, Linde A, Sleeper MM. Cardiac troponin I in feline hypertrophic cardiomyopathy. J Vet Intern Med, 2002; 16:558-564.

29. Hilgemann DW. New insights into the molecular and cellular workings of the cardiac Na+/Ca2+ exchanger. Am J Physiol Cell Physiol, 2004; 287:C1167-1172.

30. Hyun C. Valvular disease. In: Textbook of Small Animal Cardiology. Panmoon publishing, Seoul, South Korea. 2005:185-206.

31. H?ggstr?m J, Hansson K, Kvart C, Swenson L. Chronic valvular disease in the cavalier King Charles spaniel in Sweden. Vet Rec, 1992; 131:549-553.

32. Jongasaki M, Tachibana I, Luchner A. Augmented cardiotrophin-1 in experimental congestive heart failure. Circulation, 2000; 101:14-17.

33. Kangawa K, Matsumoto H. Purification and complete amino acid sequence of a human atrial natriuretic polypeptide (hANP). Biochem Biophys Res Comm, 1984; 118: 131-139.

34. Katz AM.Muscle contraction. In: Katz AM(ed.), Physiology of the heart. Lippincott

Williams & Wilkins, Philadelphia, Unite State. 2001:135-137.

35. Keffer JH. Myocardial markers of injury: Evolution and insights. Clin Chem, 1996; 105:305-320.

36. Kelly R, Struthers AD. Are natriuretic peptides clinically useful as markers of heart failure? Ann Clin Biochem, 2001; 38:575-583.

37. Kittleson MD, Kienle RD. Myxomatous atrioventricular valvular degeneration. In: Kittleson MD (ed.), Small Animal Cardiovascular Medicine. Mosby, St. Louis, Unite State. 1998:297-318.

38. Kontaraki JE, Parthenakis FI, Patrianakos AP, Karalis IK, Vardas PE. Altered expression of early cardiac marker genes in circulating cells of patients with hypertrophic cardiomyopathy. Cardiovasc Pathol, 2007; 16:329-335.

39. Labugger R, Organ L, Collier C, Atar D, Van Eyk JE. Extensive troponin I and T modification detected in serum from patients with acute myocardial infarction. Circulation, 2000; 102:122-1226.

40. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. N Engl J Med, 1998; 339:321-328.

41. Liew CC. Expressed genome molecular signatures of heart failure. Clin Chem Lab Med, 2005; 43:462-469.

42. Lobetti R, Dvir E, Pearson J. Cardiac troponins in canine babesiosis. J Vet Intern Med, 2002; 16:63-68.

43. MacDonald KA, Kittleson MD, Munro C, Kass P. Brain natriuretic peptide concentration in dogs with heart disease and congestive heart failure. J Vet Intern Med, 2003; 17:172-177.

44. Mair J. Role of cardiac natriuretic peptides testing in heart failure. Clin Chem, 2002; 48:977-978.

45. Maisel AS, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, Omland T, Storrow AB, Abraham WT, Wu AH, Clopton P, Steg PG, Westheim A, Knudsen CW, Perez A, Kazanegra R, Herrmann HC, McCullough PA; Breathing Not Properly Multinational Study Investigators. Rapid measurement of B-type natriuretic peptide in emergency diagnosis of heart failure. N Engl J Med, 2002; 347:161-167.

46. Motwani JG, McAlpine H, Kennedy N, Struthers AD. Plasma brain natriuretic peptide as an indicator for angiotensin-converting enzyme inhibition after myocardial infarction. Lancet, 1993; 341:1109-1113.

47. Ng LL, O'Brien RJ, Demme B, Jennings S. Non competitive immunochemiluminometric assay for cardiotrophin-1 detects elevated plasma levels in human heart failure. Clin Sci, 2002;

102:411-416.

48. Nielsen HB, De Palo EF, Meneghetti M, Madsen PL, Ihlemann N, Secher NH. Circulating immunoreactive pro-ANP(1?30) and pro-ANP(31?67) in sedentary subjects and athletes. Clin Chem, 2000; 46:843-847.

49. Nielsen OW, Hansen JF, Hilden J, Larsen CT, Svanegaard J. Risk assessment of left ventricular systolic dysfunction in primary care: cross section study evaluating a range of diagnostic tests. Br Med J, 2000; 320:220-224.

50. Nousiainen T, Jantunen E, Vanninen E, Remes J, Vuolteenaho O, Hartikainen J. Natriuretic peptides as markers of cardiotoxicity during doxorubicin treatment for non-Hodgkin's lymphoma. Eur J Haematol, 1999; 62:135-141.

51. O'Brien PJ, Dameron GW, Beck ML, Kang YJ, Erickson BK, Di Battista TH, Miller KE, Jackson KN, Mittelstadt S. Cardiac troponin T is a sensitive specific biomarker of cardiac injury in laboratory animals. Lab Anim Sci,1997b; 47:486-495.

52. O'Brien PJ, Dameron GW, Beck ML, Brandt M.Differential reactivity of cardiac and skeletal muscle from various species in two generations of troponin T immunoassay. Res Vet Sci, 1998; 65:135-137.

53. O' Brien PJ, Landt Y, Landerson JH. Differential reactivities of cardiac and skeletal muscle from various species in a cardiac troponin I immunoassay. Clin Chem, 1997c 43:2333-2338.

54. O' Brien PJ. Deficiencies of myocardial troponin-T and creatine kinase-MB isoenzymes in dogs with idiopathic dilation cardiomyopathy. J Vet Res, 1997a; 58:11-16.

55. Ooi DS, Isotalo PA, Veinot JP. Correlation of anti mortem serum creatine kinase, creatine kinase-MB, troponin I and troponin T with cardiac pathology. Clin Chem, 2000; 46:338-344

56. Philipson KD, Nicoll DA. Sodium-calcium exchange: a molecular perspective. Annu Rev Physiol, 2000; 62:111-133.

57. Prosek R, Sisson DD, Oyama MA, Solter PF. Distinguishing cardiac and noncardiac dyspnea in 48 dogs using plasma atrial natriuretic factor, B-type natriuretic factor, endothelin, and cardiac troponin-I. J Vet Intern Med, 2007; 21:238-242.

58. Reuter H, Pott C, Goldhaber JI, Henderson SA, Philipson KD, Schwinger RH. Na(+)--Ca2+ exchange in the regulation of cardiac excitation-contraction coupling. Cardiovasc Res, 2005; 67:198-207.

59. Roos KP, Jordan MC, Fishbein MC, Ritter MR, Friedlander M, Chang HC, Rahgozar P, Han T, Garcia AJ, Maclellan WR, Ross RS, Philipson KD. Hypertrophy and heart failure in mice overexpressing the cardiac sodium-calcium exchanger. J Card Fail, 2007; 13:318-329.

60. Schober KE, Cornand C, Kirbach B, Aupperle H, Oechtering G.Serum cardiac troponin I

and cardiac troponin T concentrations in dogs with gastric dilation?volvulus. J Am Vet Med Assoc, 2002; 221:381-388.

61. Schober KE, Kirbach B, Oechtering G. Non invasive assessment of myocardial cell injury in dogs with suspected cardiac contusions. J Vet Cardiol, 1999; 1:17-25.

62. Seiler PU, Stypmann J, Breithardt G, Schulze-Bahr E. Real-time RT-PCR for gene expression profiling in blood of heart failure patients-a pilot study: gene expression in blood of heart failure patients. Basic Res Cardiol, 2004; 99:230-238.

63. Sleeper MM, Clifford CA, Laster LL. Cardiac troponin I in normal dogs and cats. J Vet Intern Med, 2001; 15:501-503.

64. Spratt DP, Mellanby RJ, Drury N, Archer J. Cardiac troponin I: evaluation I of a biomarker for the diagnosis of heart disease in the dog. J Small Anim Pract, 2005 46:139-145.

65. Stevens TL, Burnett JC Jr, Kinoshita M, Matsuda Y, Redfield MM. A functional role for atrial natriuretic peptides in a canine model of early left ventricular dysfunction. J Clin Invest, 1995; 95:1101-1108.

66. Sudoh T, Kangawa K, Minamino N, Matsuo H. A new natriuretic peptide in porcine brain. Nature, 1988; 332:78-81.

67. Syme HM, Elliott J, Attree S. Feline plasma pro-atrial natriuretic peptide measurement: Correlation with systolic blood pressure and renal function. J Vet Intern Med, 2001; 15:302.

68. Talwar S, Squire IB, O'brien RJ, Downie PF, Davies JE, Ng LL. Plasma cardiotrophin-1 following acute myocardial infarction: relationship with left ventricular systolic dysfunction. Clin Sci, 2002; 102:9-14.

69. Turk RJ. Physiologic and pathophysiologic effects of natruiretic peptides and their implications in cardiopulmonary disease. J Am Vet Med Assoc, 2000; 216:1970-1976.

70. Uettwiller-Geiger D, Wu AH, Apple FS, Jevans AW, Venge P, Olson MD, Darte C, Woodrum DL, Roberts S, Chan S. Multicentre evaluation of an automated assay for troponin I. Clin Chem, 2002; 48:869-876.

71. Wada A, Tsutamoto T, Matsuda Y, Kinoshita M. Cardiorenal and neurohormonal effects of endogenous atrial natriuretic peptides in dogs with severe congestive heart failure using a specific antagonist for guanylate cyclase-coupled receptors. Circulation, 1994; 89:2232-2240.

72. Wu AH, Ford L. Release of cardiac troponin in acute coronary syndromes: Ischemia or necrosis. Clin Chim Acta, 1999; 284:161-174.

73. Yamada Y, Goto J, Yokota M. Brain natriuretic peptide is a sensitive indicator of impaired left ventricular function in elderly patients with cardiovascular disease. Cardiology, 1997; 88:401-407.

74. York MJ. Drug induced cardiotoxicity. Preclinical experience with cardiac troponin I. Comp Clin Pathol, 2002; 11:130.
75. Zolk O, Ng LL, O'Brien RJ, Weyand M, Eschenhagen T. Augmented expression of cardiotrophin-1 in failing hearts is accompanied by diminished glycoprotein 130 receptor protein abundance. Circulation, 2002; 106:1442-1446.
76. Moon HS, Choi E, Hyun C. The Cardiac Sodium-Calcium Exchanger Gene (NCX-1) is a Potential Canine Cardiac Biomarker of Chronic Mitral Valvular Insufficiency. J Vet Intern Med,2008 22: 1360-1365.
77. Lee JS, Pak SI, Hyun C. Evaluation of Calcium Reuptake Related Genes as a Cardiac Biomarker in Dogs with Chronic Mitral Valvular Insufficiency. J Vet Intern Med, 2009, in press.