Proceedings of the 36th World Small Animal Veterinary Congress
WSAVA

Oct. 14 - 17, 2011
Jeju, Korea

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CARDIAC BIOMARKERS IN SMALL ANIMAL PRACTICE – CAN WE DETECT HEART DISEASE WITH BLOOD SAMPLES?

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INTRODUCTION

Recent advances in veterinary diagnostic imaging technology have enabled us to detect CHF much earlier and more accurately. However, the accuracy and reliability of this technology heavily hinders the experience and skill of the operator. Furthermore, a specialist or highly trained cardiologist is required to accurately interpret the results, depict the state of a disease or prognosis. As a result, considerable research has focused on developing a more robust and easy method than diagnostic imaging technology. These studies have highlighted the importance of using cardiac biomarkers, as a reliable method for the early detection and evaluation of cardiac diseases such as chronic mitral valvular insufficiency (CMVI).

Several potential cardiac biomarkers have been identified and tested, for its suitability in the diagnosis of heart failure in dogs. Most notable of these cardiac biomarkers are: Atrial natriuretic peptide (ANP) [1], brain natriuretic peptide (BNP) [2], N-terminal proANP fragments (NT-pro ANP) [3], NT-proBNP fragments [4] and cardiac troponins (cTn) [5]. In contrast, human medical research has targeted not only the circulating biochemical markers, but the expression patterns of particular genes. This approach allows a more precise depiction of the diverse and complicated hemodynamic changes, associated with different cardiovascular abnormalities. Consequently, in conjunction with circulating biochemical markers, the regulation of candidate genes are thought to be linked to cardiac diseases; assessed via real-time RT-PCR or microarray analysis, has become more prominent in cardiology. This has also resulted in more current studies, concentrating on gene expression levels in blood; rather than tissue samples due to its noninvasiveness and accessibility. mRNA levels of disease related to genes in blood can be used as diagnostic tools for metabolic syndrome, diabetes mellitus, hypertension and congestive heart failure.

CARDIAC BIOMARKERS OF HEART FAILURE IN SMALL ANIMALS

Heart failure is the final stage of cardiac disease, which occurs due to structural and functional cardiac dysfunction. CMVI is the most common cause of heart failure in dogs, especially in small breed dogs. Although, many diagnostic imaging technologies are the current methods of detection, they are limited in that they cannot
detect the early stages of heart failure. Therefore, considerable research has been conducted to detect cardiac disease, and to estimate the degree of severity and progression of disease at earlier stages.

The importance of cardiac biomarkers, enzymes or genes related to myocardial injuries or cardiac dysfunctions, at advanced stages of heart failure have increased dramatically, even in veterinary medicine. Current cardiac biomarkers have been roughly divided into i) leakage markers related to myocardial integrity, such as cardiac troponin (cTn), myoglobin and creatine kinase isoenzyme MB (CK-MB) and ii) functional markers related to specific proteins of cardiac functions, such as brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP) and endothelin. The diagnostic values of ANP [1], BNP [2], N-terminal proANP fragments (NT-pro ANP) [3], NT-proBNP [4], and cTn [5] have been previously investigated in dogs and cats with heart failure. In human studies, cardiac biomarkers are grouped on the pathologic processes and clinical presentations, such as markers of hemodynamic stress (e.g. BNP, NT-proBNP), necrosis and ischemia (e.g. cTn, CKMB), inflammation (e.g. C-reactive protein, tumor necrosis factor-α [TNF- α]), genetic markers (e.g. Csx/ Nkx2.5, GATA4), hemostasis (e.g. fibrinogen, plasminogen activator-1) and platelet function (e.g. P-selectin) [6].

The diagnostic values of muscle leakage enzymes as cardiac biomarkers are limited due to lower tissue specificity [6]. Muscle leakage enzymes such as, CK-MB isoenzyme and cTn, have been found to be useful for detecting human acute myocardial infarction [7]. However, circulating CK-MB can also be expressed and detected in skeletal muscles, lungs, intestines, and spleen. Furthermore, CK-MB immunoreactivity is varied among species to the extent that commercial species-specific immunoassays are unavailable for dogs and cats [7].

Cardiac troponin (cTn) is a major component of the actin-myosin apparatus. Therefore, the concentrations of cTn proteins I and T in circulation are considered specific indicators of myocardial cell injury [5]. The duration and concentration of cTn in circulation is directly linked to the type and severity of myocyte injury [5]. Circulating levels of both cTn I and T are normally very low or undetectable by current assay methods. Elevated serum concentrations of cTn have been reported for dogs with CHF, hypertrophic cardiomyopathy (HCM), myocardial inflammation, and gastric dilatation/ volvulus. However, a direct correlation between cTn values and myocardial diseases or damage is not clear, because of the lack of a standardized method of analysis for dogs and cats.

Natriuretic peptides (e.g. ANP, BNP) are naturally occurring natriuretic and diuretic substances showing vasorelaxant activities. The concentration of natriuretic peptides in circulation increases in response to an increased vascular volume and decreased renal clearance. Since the N-terminal fragments of the natriuretic peptide precursors (e.g. NT-proANP and NT-proBNP) have long half-lives, tests targeting these precursors have been found to be reliable and sensitive for detecting heart failure in dogs and cats [1,2]. NT-proANP has been found to be elevated in cats with hypertrophic cardiomyopathy (HCM), myocardial inflammation, and gastric dilatation/ volvulus. However, a direct correlation between cTn values and myocardial diseases or damage is not clear, because of the lack of a standardized method of analysis for dogs and cats.

According to the human and mouse studies, many proinflammatory cytokines are known to be involved in the pathophysiology of heart failure. Cytokines that have been identified in heart diseases are interleukin IL-1, IL-6, and TNF- α. Recently, other cytokines, such as osteopontin and cardioprotrophin-1 (CT-1), have been found to be involved in the pathophysiology of heart failure [9]. TNF- α is expressed in activated macrophages and in failing cardiac tissue where it is involved in several pathological processes, such as apoptosis, myocardial fibrosis, and ventricular systolic dysfunction [6]. In human studies, TNF- α levels were found to be elevated in
THE CARDIAC SODIUM-CALCIUM EXCHANGER (NCX-1) AS A POTENTIAL CARDIAC BIOMARKER FOR DOGS

The sodium-calcium exchanger (NCX-1) is a crucial gene in cardiac excitation-contraction coupling, which is correlated to calcium (Ca²⁺) levels in cardiac myocytes [11]. Regarding the NCX-1 gene and calcium-related mechanisms of myocytes, over-expression has been observed in mice with hypertrophy and heart failure. Because this study found that the magnitude of NCX-1 expression was increased in proportion to cardiac stress by heart failure, NCX-1 may be a competent cardiac biomarker in dogs with CMVI showing signs of heart failure. Therefore, we have investigated the level of NCX-1 expression in dogs in different stages of CMVI using real-time reverse transcription polymerase chain reaction (RT-PCR) to evaluate the diagnostic value of NCX-1 as a cardiac biomarker for canine CMVI [12]. This study revealed that the fold differences in the levels of mRNA expression compared to controls were found to be 1.39 ± 0.88 in New York Heart Association (NYHA) Group I, 1.32 ± 0.65 in NYHA Group II, 4.86 ± 1.25 in NYHA Group III, and 5.96 ± 1.69 in NYHA Group IV. The expression of NCX-1 was significantly increased in NYHA Groups III to IV (P < .05), while expression levels in NYHA Groups I to II were insignificant compared to healthy controls. The level of NCX-1 expression was also found to be increased significantly, in the moderate to severe CMVI Groups. These animal studies, highlight the potential of NCX-1 as an alternative cardiac biomarker; NCX-1 was also demonstrated to be specific for heart failure from CMVI [12].

Since the expression levels of some cardiac biomarkers have been known to be influenced by renal failure, this limits their reliability as cardiac biomarkers in patients that also have renal disease. NCX-1 is expressed in kidneys and thus may also be elevated in the peripheral blood of animals with renal failure or azotemia. Consequently, this may significantly decrease the diagnostic value of NCX-1 in dogs with CMVI, however, there has been no evidence demonstrating that this is the case. Therefore, we also evaluated the effect of azotemia on NCX-1 expression level in dogs with respect to CMVI. NCX-1 expression was significantly elevated in moderate (2.99 ± 0.61) to severe (4.35 ± 1.44) CMVI dogs (p < 0.01). In contrast, NCX-1 expression was not elevated in the azotemic dogs. Furthermore, there was also no correlation between the increased level of serum renal enzymes (creatinine and urea nitrogen) and NCX-1 expression in the renal failure Group. The study showed that azotemia likely did not affect NCX-1 expression [13].

direct correlation with the severity of CHF in patients [6]. Recently, CT-1 levels were reported to be linked to the level of hypoxia and reperfusion injury [9]. Interestingly, an increase in CT-1 plasma levels often preceded hypertrophic changes in the myocardium of animals and humans with heart failure. Osteopontin is also known to be elevated in humans with heart failure with respect to the New York heart association (NYHA) classification [10].
References