Proceeding of the ACVP/ASVCP
Concurrent Annual Meetings
November 10-14, 2007
Savannah, Georgia

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Clinical Biomarkers of Cardiac Injury and Disease

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Introduction

Human diagnostic medicine began developing blood biomarkers for heart disease in the 1950s. Typically, veterinary medicine is quick to take advantage of progress made in human medicine. However, it wasn’t until nearly the beginning of this century that cardiac biomarkers had evolved to a point where tests of potential value to veterinary species were discovered. With these advances, prospects are improving that tests for the routine screening and monitoring for progression of cardiac injury and disease will be added to the veterinary clinical chemistry armamentarium. Several clinical validation studies are ongoing, and there is a slowly growing body of evidence suggesting that the potential benefits of blood tests for heart disease in veterinary species are similar to those in human medicine. Several automated human immunoassays appear to cross-react and have appropriate sensitivity for the diagnosis of heart diseases common to veterinary medicine. Another exciting development is the availability of veterinary-specific commercial test kits for cardiac function. Ultimately it may be shown that these tests improve overall diagnostic accuracy while conserving costs and offering a less invasive and relatively simple alternative to other diagnostic modalities. This presentation will give a brief overview of classic and novel biomarkers of cardiac injury and dysfunction that can be applied by the clinical pathologist, and briefly reviews the advances that have occurred in this area which make this an interesting time for veterinary cardiac biomarker research.

A brief history of cardiac biomarkers

The earliest blood biomarkers of cardiac injury and disease were activity-based assays to cytosolic myocardial enzymes. These included aspartate aminotransferase (AST) which was the first, lactate dehydrogenase (LDH), and creatine kinase (CK). Beginning in the 1950s, these tests were added to the expanding collection of rapid, automated clinical chemistry assays. These enzymatic assays were found to be of most use as screening tests for ischemic myocardial necrosis brought about by acute myocardial infarction (AMI). These first-generation tests lacked cardiac tissue specificity, being present also in skeletal muscle and other tissues. They furthermore lacked sensitivity, with relatively high baseline values that made interpretation of small increases in serum enzyme activity difficult. These older enzymatic assays performed even worse when used to screen for non-ischemic diseases. Regardless, the growing recognition after World War II of the importance to human health of coronary heart disease made these assays of clinical value.

An important advance in cardiac biomarkers was the invention and use of monoclonal antibody strategies in the 1980s and subsequent automated immunoassay techniques. Coming from this improvement was the antibody based CK-MB assay and other tests. This began a new generation of cardiac biomarkers that were antibody-based, and resulted in an immediate improvement in the diagnosis of AMI. Again, however, these early immunoassays were still of inadequate sensitivity to be of value for more chronic heart diseases that do not cause substantial myocardial necrosis, such as valvular insufficiency, septal wall defects, and cardiomyopathies. Given that myocardial infarcts are rare in veterinary species, veterinary clinical pathologists did not benefit greatly from these first human immunoassays. Moreover, the reliance of these assays on accurately antigen-antibody interactions inadvertently added a level of complexity to their use in veterinary medicine. On the other hand, the switch from activity assays to immunoassays opened a whole new world of potential nonenzymatic biomarkers for investigation. This was ultimately the key to the discovery of sensitive blood tests of cardiac function and injury with potential value to veterinary medicine.

Biomarkers of contemporary interest in human and veterinary medicine

The assessment of cardiovascular health by clinical chemistry in humans includes at least 4 types of clinical conditions: myocardial injury/necrosis, myocardial (i.e., left ventricular) function, serum lipoprotein homeostasis, and inflammation of the cardiovascular system. The current state of the art of clinical biochemistry testing for these disease conditions is respectively, cardiac troponins, plasma B-type natriuretic peptide (BNP), serum lipoprotein/cholesterol profiles (HDL, LDL, etc.), and serum C-reactive protein (CRP). Automated assays exist for each, although the clinical value of some has yet to be fully worked out. While occasions arise in veterinary medicine for lipoprotein and CRP monitoring in veterinary medicine, they are not specific to myocardial disease and therefore will not be considered further here. In contrast, BNP and troponins could be of significant use as heart disease biomarkers in veterinary clinical pathology. Moreover, recent studies suggest...
that these new cardiac biomarkers may be of value as prognostic indicators of cardiac morbidity and mortality and risk stratification, not only prior to beginning heart failure treatment, but after the initiation of treatment.15

**Troponins**

Troponins are components of the contraction apparatus of striated muscle cells. There are three distinct troponin proteins, TnC, TnI, and TnT. They each serve different functions within the contractile complex, and vary in size from TnC at 18 kDa, to TnI at 22 kDa, and TnT at 37 kDa. For TnI and TnT, there are genetically distinct cardiac isoforms, cTnI and cTnT. Both cTnI and cTnT are believed to be produced only in cardiomyocytes, which is the basis for their presumed high tissue specificity and use as cardiac biomarkers. In contrast, there is only one genetic form of TnC, which is therefore not used in troponin immunoassays.

There is a great deal of homology between human and nonhuman cardiac troponin isoforms.30 Because of this fortuitous situation, many automated commercial human cardiac troponin immunoassays can be used in nonhuman species. The commercially available cTn immunoassays thus far tested include, the Biosite Triage Meter, the Dade-Behring Stratus, and the Beckman-Coulter Access AccuTnI.1 The results from studies using both canine cTnI spiked canine plasma and plasma from dogs with cardiac disease correlate well. However, the apparent recovery of canine cTnI by the different assays varies dramatically, suggesting perhaps differences in the matrix effect of canine plasma in each assay, in the affinity of the anti-human cTn antibodies between assays, or differences in test calibrators. Such findings demonstrate the fact that serious laboratory validation of these assays for use with canine plasma has yet to be done. Studies of cTnT are less common, perhaps due to the relatively small number of automated cTnT assays available commercially.14

Troponins have been called the “biomarker of choice” for cardiac injury of humans, supplanting CK-MB for the diagnosis of acute myocardial infarction (AMI).3 Their enhanced sensitivity and specificity is likely the reason why cardiac troponins have been found effective in veterinary species. The progressively increasing sensitivity of each generation of troponin assays makes them potentially sensitive biomarkers of ongoing myocardial damage from both primary cardiac and noncardiac disease, such as canine dilated cardiomyopathy which causes a mild increase of cTnI.28 Fewer studies have been done in cats, however the results suggest that cTnI may have some value in the diagnosis of feline hypertrophic cardiomyopathy8 and that cTnI roughly reflects disease severity.13 It may also have a role in determining the response to treatment of feline hyperthyroidism.9 While cTnI may be capable of detecting diseases associated with primary cardiac dysfunctions and low-level myocardial necrosis, head to head comparisons with other cardiac biomarker assays show them to have less diagnostic accuracy than natriuretic peptides.28,29 Hence, their strongest clinical role may be in gauging and monitoring the severity of cardiac damage from insults such as trauma or infections, and for risk stratification and response to therapy.23

**The natriuretic peptides**

Of the currently available tests, the natriuretic peptides may have the most potential as clinical biomarkers of cardiac disease in veterinary species. Three natriuretic peptides have been described in mammals, Atrial or A-type (ANP), Brain or B-type (BNP), and C-type (CNP). Their physiological role is to help maintain cardiovascular homeostasis by increasing natriuresis, decreasing arterial blood pressure, and inhibiting the renin angiotensin aldosterone system.24 A fourth type, DNP, was discovered in venom from green mamba snakes that, conveniently for naming purposes, belongs to the genus *Dendroaspis*. Both ANP and BNP are produced by cardiomyocytes. In contrast, CNP is produced by endothelial cells, has primarily a paracrine function, is involved in bone growth, has low peripheral blood concentrations, and is not currently considered of value as a biomarker of myocardial function.25 Therefore, the majority of emphasis in heart biomarker research has been on the clinical utility of ANP and BNP.

Both ANP and BNP are synthesized as preprohormones consisting of a signal sequence and prohormone segments27 (Figure 1). The signal sequence is cleaved from the prohormone portion following appropriate intracellular migration. At, or immediately following secretion of the prohormone into the blood, the peptide

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**Figure 1.** Basic structure and nomenclature of the natriuretic peptides (NP), either ANP or BNP.
is cleaved into an inactive N-terminal fragment (NTproANP or NTproBNP) and a physiologically active C-terminal segment (ANP or BNP). The size of each ANP segment is relatively similar between species, with an NTproANP fragment of 98 amino acids and an ANP fragment of 28 to 30 amino acids. The NTproBNP and BNP segments vary more between species than is true for ANP. Canine NTproBNP and BNP are 82 and 32 amino acids, respectively. In all species, the C-terminal domain of ANP and BNP contains a 17-amino acid ring, formed by a cys-cys disulfide bond, that is important for each peptide’s physiological activity.

Both ANP and BNP are released from the heart in response to myocardial stretching, induced usually by increased cardiac chamber wall pressures. proANP is constitutively produced and stored within granules in the atrial myocardiocytes, along with lesser amounts of proBNP. Under normal conditions proBNP is constitutively produced in small amounts by ventricular myocardiocytes and released without storage. Production of proBNP increases greatly with ventricular dysfunction. Some ventricular synthesis of ANP also occurs with heart disease; however, the amount is much less. ANP and BNP are cleared from the blood by natriuretic peptide receptors on sensitive cells throughout the body, and degraded by neutral endopeptidase in the kidney and perhaps other organs. The plasma half-life of BNP, measured in humans, is roughly a short 22 minutes. Similar clearance mechanisms are not believed to exist for NTproANP and NTproBNP. As a result, their plasma half-lives are believed to be longer, and their plasma concentrations higher and more stable than their C-terminal counterparts. This may make measuring the NTproNP fragments technically simpler than the C-terminal peptides.

Although commercial blood tests to human ANP, BNP, NTproANP, and NTproBNP have all been available in the past, only BNP and NTproBNP assays have been extensively automated. In fact, some ANP and NTproANP bench assays have been discontinued. Comparisons of diagnostic performance in humans have revealed that BNP and NTproBNP are superior heart failure biomarkers, which is likely the reason for the higher clinical interest in B-type natriuretic peptides. The emphasis on development of automated BNP and NTproBNP assays, at the exclusion of comparable ANP assays, is perhaps unfortunate from the standpoint of a veterinary clinical pathologist, because the interspecies homology of proANP is much greater than that of proBNP. Indeed, sequence matching using the NCBI BLAST system reveals 88% and 92% amino acid sequence alignment of human proANP with canine and feline proANP, while similar matching of human proBNP with canine and feline proBNP shows only 46% and 47% sequence homology. Hence, while several human ANP assays have been shown to cross-react with canine and feline natriuretic peptides, human BNP and NTproBNP assays are not co-antigenic with the analogous feline and canine peptides. This has meant that veterinary medicine has not been able to use many of the automated natriuretic peptide assays, which are almost universally superior to bench top procedures in accuracy, precision, and speed of analysis.

Several studies have tested the clinical performance of BNP, ANP, and NTproANP in veterinary medicine. The results are encouraging and suggest that natriuretic peptides may play several roles in the workup of suspected heart failure cases. For example, as in man, they may be advantageous in discriminating acute dyspnea due to congestive heart failure from other causes, and increase with asymptomatic cardiomyopathies. A great deal of work however remains to be done. For instance, the B-type natriuretic peptide assays are proving capable of playing a role in human medicine in the selection and monitoring of heart failure therapy. This would be an extremely valuable aspect of BNP/NTproBNP monitoring that has not been thoroughly explored in veterinary medicine. Another exciting development is the very recent availability of ELISA kits to canine and feline NTproBNP from Guildhay, Ltd. (www.guildhay.co.uk). This has removed a tremendous obstacle to the performance of clinical validation studies of NTproBNP in dogs and cats. Interestingly, very recent evidence supports a long held suspicion that uncleaved proBNP may be present in the peripheral blood. In fact, it has been suggested that blood BNP assays are actually measuring proBNP rather than the active peptide. This may in part explain the fact that only slight differences in diagnostic accuracy are frequently found in head-to-head comparisons of plasma BNP and NTproBNP in human medicine.

**Novel and future biomarkers of cardiac injury and dysfunction**

One expansion in the future of cardiac biomarkers will likely be in developing earlier, more sensitive assays that may detect myocardial ischemia before complete myocardial infarction. Several of these advances may benefit veterinary medicine as well. For example, the possibility to test for evidence of cardiac ischemia may increase the ability to stratify survival risk and response to therapy in veterinary patients. Several of the candidate myocardial ischemia markers are cytosolic proteins. One such protein of particular interest is heart-specific fatty acid binding protein (hFABP). Recently it has been shown that hFABP may be more sensitive than troponins in monitoring ongoing myocardial damage in heart failure patients. The amino acid sequence of hFABP is relatively well conserved among species, which may offer the possibility of interspecies immunoassays. Additional progress has been made...
recently in the development of products of altered metabolism as cardiac biomarkers. Whole blood choline concentrations, which are released from membrane phospholipids, and unbound free fatty acids released from cardiac tissue with ischemia have been proposed, although the analysis techniques are only amenable to basic research at this time. One assay along these lines however that is undergoing clinical testing is the measurement of ischemia modified albumin (IMA). This is actually an albumin-cobalt binding test. The basis of the test is the fact that ischemia produces a decrease in the metal binding capacity of serum albumin. Studies have shown that IMA can detect myocardial ischemia before the occurrence of myocardial infarction. Such an assay would be of great benefit in averting myocardial damage. An assay with such sensitivity might also allow the detection of cardiac ischemia from causes other than coronary artery disease, and therefore be of benefit in veterinary medicine along similar lines as the cardiac troponin assays.

Conclusions

The future of clinical biomarkers of cardiac injury and disease is encouraging. Veterinary medicine is now able to take advantage of the advances that have been made in biomarker development to a degree that was not possible earlier. While it is always interesting to imagine what potential tests are down the road, the current set of tests, and especially the introduction of natriuretic peptide assays, makes it highly likely that veterinary clinical biochemistry will soon be able to play a role in the diagnosis and monitoring of cardiac disease and injury as it has in diseases of other body organs, such as the liver, kidney and pancreas.

References


