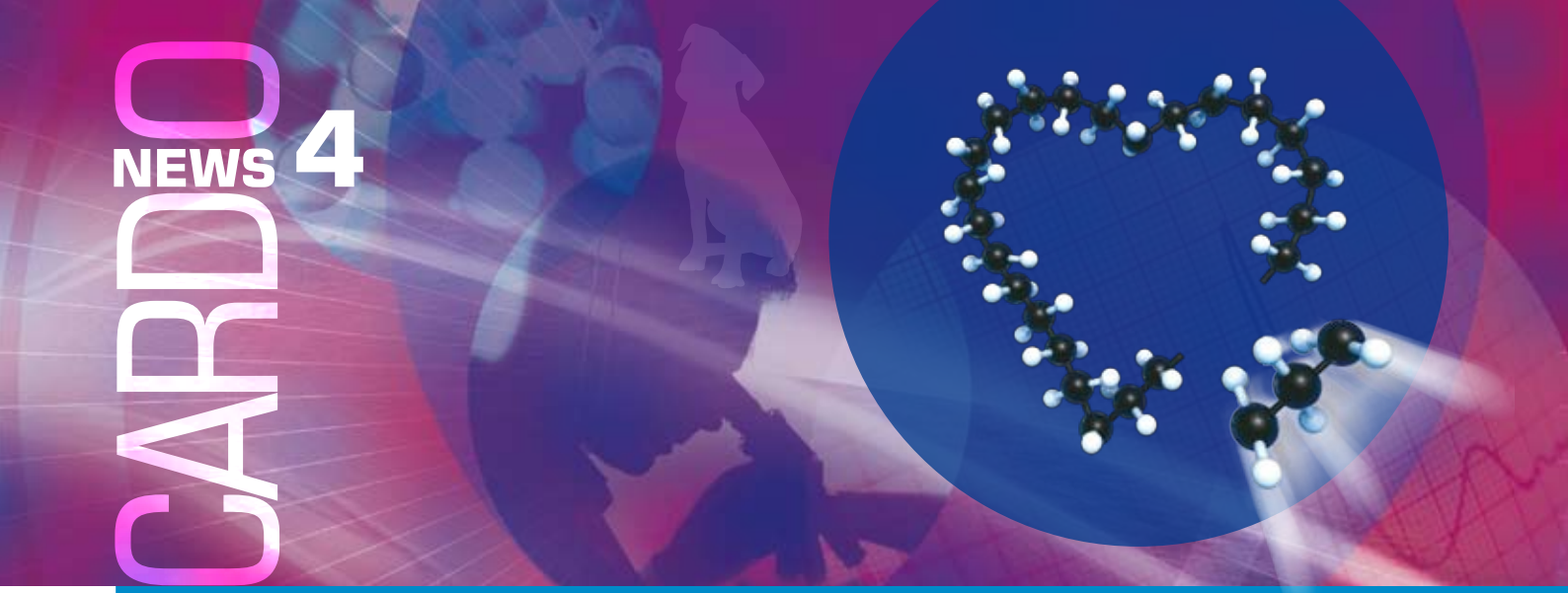

CARDIO NEWS 4

CEVA SANTÉ ANIMALE | AT THE HEART OF INNOVATION

Cardiac biomarkers

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Keywords: biomarkers, diagnosis, heart disease, heart failure, troponin, NT-proBNP

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Today cardiac biomarkers are a promising new tool for the diagnosis of cardiac disease in dogs and cats and, throughout this issue, Adrian Boswood shares his thoughts and experiences on the subject.

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What is a biomarker?

One useful but very broad definition is that a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”.[1]

This definition could include a multitude of measureable factors. Increasingly the definition is used in a more restricted way to describe markers of disease that are usually measured in blood and are not measured as part of a routine biochemical or haematological profile. There are a great many circulating markers which can be altered by the presence of cardiovascular diseases[2], but currently in human patients only two markers are considered sufficiently well validated for them to have been widely adopted into medical practice guidelines.

These are the troponins as indicators of myocardial infarction[3] and natriuretic peptides for patients with left ventricular dysfunction and heart failure.[4] Other types of markers that have been described as being valuable in patients with cardiovascular disease include markers of endothelial dysfunction, markers of inflammation and markers of remodelling.[2] Although many of these have also been researched to some extent in veterinary patients, their clinical value has yet to be clearly demonstrated and they are not currently available in any setting other than as research tools.

Troponins

• The troponins I and T are intracellular proteins that, with currently available assays, are not present in detectable concentrations in the circulation of the majority of normal dogs.[5] Damage to myocardial cellular integrity, brought about by ischaemia, infarction, inflammation or degenerative disease, results in the leakage of troponin into the extracellular space and hence into the plasma where it can be detected (Figure 1).

The types of heart disease that result in significant simultaneous cell death are relatively rare in dogs and cats, although ischaemic heart disease is recognised in veterinary patients.[6] There have been many articles regarding the assessment of troponin concentrations in veterinary patients[7-10], but their potential as markers for outcomes of most interest to veterinary practitioners does not appear to be as great as that of the natriuretic peptides.[11]

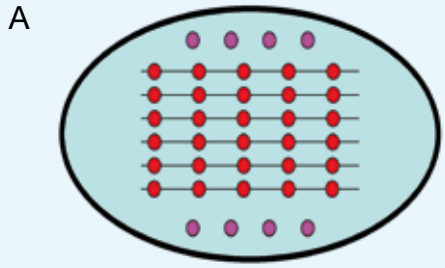

Figure 1(A). *In normal myocardial cells troponin exists in the cytosol (purple circles) and bound at regular intervals to the actin strand of the contractile proteins (red circles). There is no appreciable amount of troponin in the extracellular region.*

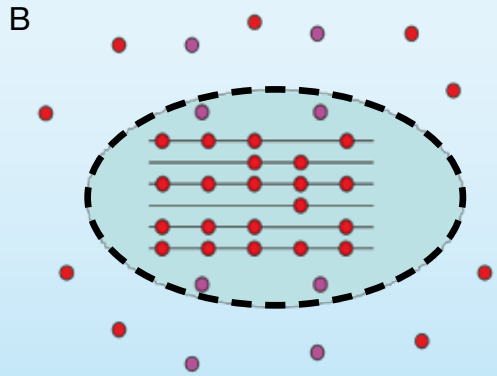

Figure 1(B). *Loss of integrity of the myocardial cells results in troponin loss into the extracellular space, and hence into the plasma, from both the cytosolic compartment and release from the myofibrillar proteins.*

Natriuretic peptides

• Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are manufactured by the atrial and ventricular myocardium. They are produced in greater quantities in response to increased myocardial stretch and increased wall stress. Their concentrations therefore increase when cardiac filling pressures are increased; a feature integral to the development of congestive heart failure. They are initially manufactured as large pro-hormone molecules from which the C-terminal is cleaved to make the active peptide (Figure 2).[12] The N-terminal fragments, largely considered to be inactive by-products of manufacture of the C-terminal peptide, are released in equal quantities and therefore measurement of the N-terminal peptides provides useful information about the activity of the peptide systems in general. Although both the C-terminal and N-terminal peptides can be measured for both ANP and BNP there are advantages to measuring the N-terminal fragment; it has a longer half-life, which results in plasma concentrations being higher and less prone to fluctuation.

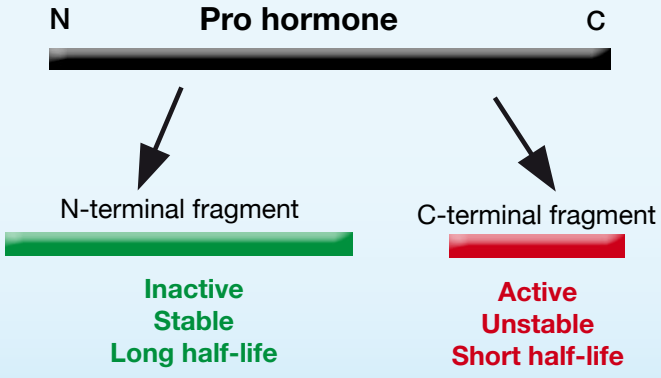

Figure 2. *Schematic diagram illustrating the manufacture of natriuretic peptides. Cleavage of the C-terminal portion from a larger pro-hormone results in the creation of two peptides; the active C-terminal fragment and the residual N-terminal fragment.*

• A recent review article concluded that, for human patients, clinical uses of natriuretic peptide measurements (NP) include them being quantitative biomarkers of heart failure (HF), accurate diagnostic tests for the presence of HF, useful for risk stratification, able to improve patient management and reduce total treatment costs in patients with acute dyspnoea and predictors of death and re-hospitalisation in HF patients. It has even been proposed that, when used to guide therapy, they may improve morbidity and/or mortality in heart failure patients.[13] Given this multitude of potential uses, it is not surprising that the development and introduction of this completely new way of evaluating patients with heart disease has been met with excitement, scepticism and opposition in equal measure.

• Research regarding the use of natriuretic peptides in veterinary patients is at an earlier stage, but developing along similar lines, with studies suggesting the measurement of these markers may have similar promise in veterinary species. There are many different types of natriuretic peptide that can be measured. The main ones that have been studied in veterinary patients are BNP[11,14], ANP[15], proANP, proANP fragments[16] and NTproBNP[17-20] and their concentrations have all been shown to increase with increasing severity of cardiac disease. Figure 3 illustrates concentrations of NTproBNP in dogs with heart disease and heart failure compared to those with respiratory disease (See reference 17 for full details). Increased concentrations are associated with the presence of heart disease, with even greater elevations in dogs showing signs of heart failure.

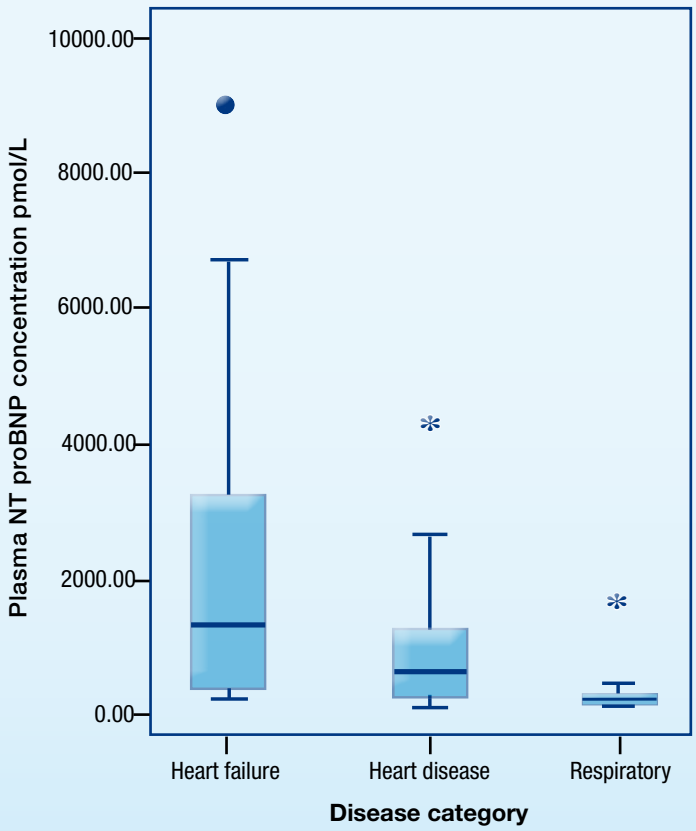

Figure 3.

NTproBNP concentrations in canine patients according to their underlying disease. Patients with heart failure have higher concentrations than those with heart disease which in turn have higher concentration than dogs with respiratory disease. The bar represents the median concentration for the group, the whiskers represent the range of measurements and the circle and stars represent outliers. (from Boswood et al., 2008 [17]).

• A few studies have compared the relative value of these markers in the diagnosis of heart failure. Two studies have suggested that NTproANP is more effective than BNP at discriminating dogs with heart failure from those without[11,14], whereas another study suggested that NTproBNP was more effective than proANP fragments at distinguishing patients with cardiac disease from those with respiratory disease.[17] Although the genuine supremacy of one test over another cannot claim to have been established beyond doubt, recent interest has centred on the evaluation of NTproBNP in both dogs and cats.[17-20] This is partly for the very pragmatic reason of the availability and ease of use of the assay. In order to be of value to clinicians, one of the critical factors to consider is the availability of the assay[21] and the measurement of NTproBNP is now widely available through a commercial diagnostic laboratory[i].

How do we assess the value of natriuretic peptides?

• Currently published studies on natriuretic peptides are predominantly cross-sectional, where the ability of the test to discriminate between patients with or without a particular condition is determined. In these studies assays are often described in terms of their sensitivity and specificity and their overall "accuracy". This is a simple and useful way of describing the performance of these tests, but the data tend to be derived from relatively restricted populations of patients where some animals with concurrent disease have been excluded. Concentrating on NTproBNP, the studies that have so far described the diagnostic value of this test have produced apparently conflicting results. This is probably a consequence of the studies being conducted slightly differently and the samples used in the studies being handled differently. Fine and others[19] suggested that 92% of dogs with heart failure had an NTproBNP concentration above 1,400 pmol/L. Oyama and others[20] suggested that 83% of dogs with cardiac disease have concentrations over 445 pmol/L (sensitivity of 83% for the detection of heart disease) whereas 90% of normal dogs did not (specificity of 90%). In the latter study a value of 820 pmol/L was suggested as a threshold beneath which a patient was very unlikely to have heart failure. Finally in a study we conducted[17] a lower cut-off for the detection of heart disease was suggested at 210 pmol/L, although the majority of dogs with heart failure had concentrations over 1000 pmol/L. The lower cut-off value in our study was probably a reflection of less rigorous sample handling. If plasma is separated from the cells within a few hours and frozen before analysis, higher values are likely to be obtained because of less degradation of the peptide. In a more recent (currently unpublished) study handling samples more carefully, we obtained values from twenty normal relatively elderly small breed dogs (median age of 10 years). The median concentration in this population was approximately 400 pmol/L (W.Moonarmart personal communication) confirming the previously suggested cut-off of 210pmol/L to be too low.

• There are currently fewer papers in the literature regarding natriuretic peptides in cats but the diagnostic ability of species specific assays, in a species in which it is typically difficult to make a conclusive diagnosis of heart failure, appears to be excellent.[18]

• In reality the plasma concentration of NTproBNP is likely to increase with increasing severity of cardiac disease hence the fact that it is referred to as a "quantitative" (as opposed to qualitative) biomarker of heart failure.[13] This means increasing concentrations are associated with an increasing likelihood of the presence of more advanced cardiac disease and concentrations around the proposed "cut-off" values are always going to be those which are the most difficult to interpret. Figure 4 illustrates a slightly more sophisticated view of a diagnostic test result. If the likelihood of the abnormality of interest, in this case the presence of heart failure, increases as the concentration of the marker increases then at low concentrations the likelihood of the patient having a disease is low and the likelihood of the patient being normal is high. As the concentration of the marker increases the likelihood of the patient being normal diminishes whereas the likelihood of the patient being diseased increases.

At some point the values for likelihood will cross i.e. there is a concentration at which the patient is equally likely to be normal and abnormal. Beyond this point, as

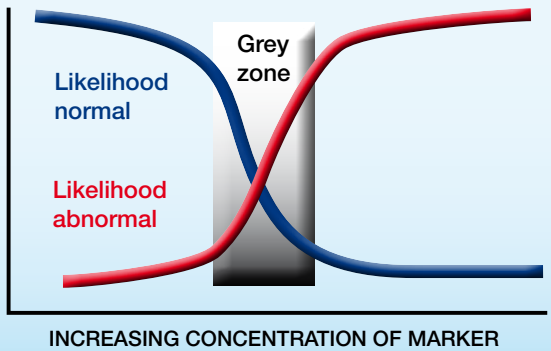

Figure 4. *Schematic illustration of the effect of increasing concentrations of a biomarker on the likelihood if disease being present.*

the concentrations continue to increase, the patient is more likely to be diseased than normal, and the higher the numerical value the more likely it is that the patient is abnormal and the less likely it is that they are normal. It is useful therefore to interpret values in the intermediate area, where likelihoods of normality and abnormality are roughly similar, with a suspension of certainty i.e. we should be comfortable with a grey zone where the results do not definitely tell us if the patient is normal or not. In the case of using NTproBNP to assess dogs for the presence of significant cardiac disease this grey zone is probably between about 445 and 1000 pmol/L. Values in this range should not be interpreted in isolation but rather taken in conjunction with other data, before any definite decision is taken about a particular case.

Other potential uses for natriuretic peptides

As was mentioned above, the majority of studies so far published regarding biomarkers in veterinary patients have evaluated their potential as diagnostic markers in cross-sectional studies. Comparatively little has been published describing their use in prediction of prognosis, response to therapy or guiding therapeutic decision making. In human patients the prognostic potential of natriuretic peptides and troponin concentrations are well established[22]. There are numerous studies where reduction of concentrations of markers in response to therapy have been described and recently interest has centred on the potential for concentrations of natriuretic peptides to aid with the guidance of therapy, resulting in improved outcomes for patients.[23, 24] Some veterinary studies have suggested the potential for biomarkers such as troponin[9] and natriuretic peptide concentrations[25, 26] to act as indicators of prognosis. Finally, the reduction of natriuretic peptide concentrations in response to the introduction of treatment for congestive heart failure has also been demonstrated in dogs[27], but their potential as monitoring tools or methods of guiding therapy has yet to be explored.

WHAT WILL THE FUTURE BRING?

The development and evaluation of biomarkers in both human and veterinary medicine remains a very active area of research. We have yet to fully explore the potential of the currently available markers and it is very likely that different markers, or combinations of markers, will become available in future. The challenge for the researchers in this field is to demonstrate whether or not these markers have real value in routine clinical practice. This could be achieved in various ways; firstly value could be established through demonstrating them to be independent markers of diagnosis and prognosis, providing clinicians with information more accurately, more easily, more conveniently, more cheaply or more quickly than currently available methods. In order to overcome the scepticism and opposition, that is the natural reaction of practitioners and cardiologists when a new test becomes available, ultimately what needs to be shown is that the standard of care that veterinarians can offer is improved through use these markers. This would be most conclusively demonstrated if it was possible to show that outcomes for patients are improved when these markers' concentrations are known. This requires well constructed longitudinal studies measuring relevant outcomes in patients with common diseases. There is plenty of work to be done!

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