Biochemical Markers of Cardiac Disease (Biomarkers)
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Introduction

A **Biomarker** is any characteristic that can be evaluated as an indicator of normal or pathological processes. Blood-based biomarkers can provide information about the presence and severity of disease, and may also offer some prognostic value. We can broadly classify biomarkers into leakage markers and functional markers.

Leakage markers are intracellular components which are released as a result of cell death or loss of cell membrane integrity (e.g. ALT & AST- markers of hepatocellular damage; Troponin I-marker of cardiomyocyte damage). Pressure overload, hypoxia, ischemia, toxins, calcium abnormalities, and inflammatory cytokines can cause an increase in cell damage, and thus, an increase in leakage markers.

Functional markers indicate organ function and response to disease (e.g. BUN- renal function; NT-proBNP- myocardial stretch/dysfunction). These biomarkers can be used to diagnose, characterize and prognosticate disease, as well as to evaluate response to therapy.

Potential Clinical Uses

- Screening of breeding populations
- Early disease detection in at-risk populations; high sensitivity allows for earlier intervention, reducing morbidity and mortality
- Detection of myocarditis, myocardial infarction, pulmonary hypertension
- Diagnosis of CHF and differentiation of heart failure from primary respiratory disease.
- Prognostic information
- Monitoring disease progression over time.
- Monitoring effects of cardiotoxic drugs or cardiac effects of systemic disease

I. Cardiac Leakage markers

Troponins

- **Structure and function:** The cardiac troponin (cTn) complex contains three subunits located on the thin (actin) filament of the contractile apparatus where it mediates excitation-contraction coupling
    - **Troponin-I** (**cTnI)** exerts an inhibitory influence on cardiomyocyte contraction by preventing actin-myosin interaction until cTnC is bound by intracellular calcium. cTnI is specific to the heart and serves as a highly sensitive and specific indicator of myocardial cell necrosis, making this biomarker a very useful diagnostic tool.
    - **Troponin-T (cTnT)** is responsible for binding the troponin-tropomyosin complex to the actin filament. Like cTnI, cTnT has distinct skeletal and cardiac isoforms.
    - **Troponin-C** (**cTnC**) binds intracellular calcium. Skeletal and cardiac cTnC isoforms are identical, which makes this biomarker non-specific, (i.e. cTnC measurements are of no practical value).
- **Troponin release and metabolism**

    - Cardiac troponins can be found in one of two pools within the cardiomyocyte: the larger structurally bound pool, and the smaller free cytosolic pool. Cardiomyocyte necrosis, compromised membrane integrity, and cardiac sarcomere injury cause the release of cTn into the cytoplasm, then subsequently into the interstitium. Serum levels of cTn are very low, nearly undetectable unless a massive release has overwhelmed lymphatic clearance of the troponins.
    - Detectable cTn levels are affected by:
        - Release from cardiac myocytes
        - Leakage into the general circulation
        - Degradation by serum proteases
        - Clearance by the kidney, liver, and reticuloendothelial system.
- **Troponin assays**
    - The structure of cardiac troponin isoforms is closely preserved among species. This lack of species-specificity allows veterinarians to utilize troponin assays that were originally developed for humans. These assays do not cross-react with the skeletal troponin isoforms. It is important to note that reference ranges vary widely from one analyzer to another.
    - In healthy dogs and cats, troponin levels are usually very low, however age, strenuous exercise and non-cardiac disease can cause minor elevations in cTnI.
    - Both cTnI and cTnT are highly specific for myocardial damage, but measurement of cTnI is generally preferred because it becomes elevated earlier than cTnT, and it has been more widely reported in the veterinary literature. While detection of these molecules can alert you to the presence of cardiomyocyte necrosis, troponin levels cannot indicate whether this damage is due to CHF, trauma, toxins, neoplasia, etc.
- **Clinical Applications**
    - Detection of CHF in cats
        - While canine cTn levels cannot be reliably used to detect congestive heart failure, feline cTn levels **do become elevated** in the majority of cats with CHF. Elevations in cTn are seldom used as a stand-alone test for CHF, but they can be used as additional supportive evidence of heart failure vs respiratory disease as the cause of dyspnea in cats.
    - Monitoring cardiotoxic drugs
        - Doxorubicin is a chemotherapeutic agent that has known cardiotoxic effects. Measuring serial cTn levels after doxorubicin therapy may allow earlier detection of cardiotoxicity and help guide therapeutic decisions.
    - Pericardial effusion in dogs
        - Levels of cTnI are elevated in dogs with pericardial effusion. Levels of cTnI are even further elevated in dogs with effusion resulting from hemangiosarcoma compared to dogs with idiopathic or other causes of pericardial effusion.

II. Cardiac Functional Markers

Natriuretic Peptides

- **Structure and function:** The Natriuretic peptides are release in resonse to stretch or hypertrophy of the myocardium, and they exert potent natriuresis, diuresis, and balanced vasodilation by counteracting RAAS, the sympathetic nervous system, and vasopressin release.
    - Net effects: decrease salt and water retention, decrease blood pressure and attenuate cardiomyocyte hypertrophy and fibrosis.
  - **Atrial natriuretic peptide** is predominantly produced by the atria where it is synthesized as pre-proANP, then cleaved into pro-ANP, which is stored as granules in atrial myocytes. Pro-ANP is subsequently cleaved into inactive NT-proANP and active C-terminal ANP, which are released into the blood in response to atrial stretch and dilation.
    - ANP granule release is proportional to the degree of atrial stretch or increased heart rate. Because the granules are stored, ANP levels can increase rapidly, making ANP a marker of acute atrial distension.
  - **Brain natriuretic peptide,** is produced in low levels in a normal animal; however, in the case of chronic pressure or volume overload (increased wall stress), the ventricles become the major source of BNP. Pre-proBNP is synthesized and processed into active BNP and inactive NT-proBNP. NT-pro BNP has a longer half-life and higher serum concentrations than BNP, and there have been more veterinary studies reporting the clinical uses of NT-proBNP, making NT-proBNP more clinically useful.
    - BNP synthesis is controlled at the level of transcription, so that a longer term stimulus is needed to cause BNP release. Practically, this means that BNP is less susceptible to rapid changes in hemodynamic status.
- **Natriuretic peptide assays**
  - Commercial assays for the natriuretic peptides are not standardized, so each analyzer may produce markedly different numbers with different reference ranges.
  - ANP assays are not species specific, but BNP assays are, meaning human assays cannot be used to analyze BNP in veterinary patients. It is also important to be aware that some labs choose to measure BNP, while others measure NT-proBNP. A cage-side snap test is available for NT-proBNP in cats, but a similar test is not yet available for dogs.
- **Clinical Applications of NP levels**
    - Elevated NP levels are associated with volume overload states (CHF), ventricular hypertrophy, decreased renal clearance, tachypnea, and hypoxia. NP levels will increase in a variety of conditions, including chronic valvular disease, dilated cardiomyopathy (DCM), aortic stenosis, and heartworm disease. Measurement of natriuretic peptide levels can aid in differentiating cardiac from primary respiratory disease and can also be utilized as a screening tool for at-risk or breeding populations (ie Doberman Pinscher).
      - **NT-proBNP** is a sensitive and specific marker for the detection of cardiomyopathy and valvular disease. Levels correlate with the degree of

LV dysfunction, increasing heart size, and worsening CHF class, making NT-proBNP helpful in predicting how close dogs are to developing CHF

Other Potential Functional Markers

- There are several other molecules which have been flagged as potentially helpful in the assessment of cardiac function. These markers are not currently utilized but may become more clinically useful in the future:
    - Endothelin
    - Cytokines & miscellaneous markers
        - TNF-α
        - C-reactive protein (CRP)
        - Nitric Oxide (NO)
        - Adrenomedullin

	Action	Release Stimulation	Disease Processes Indicated
Endothelin (peptide)	Potent vasoconstrictor Inotropic effects Mitogenic effects	pulsatile vessel stretch Low shear stress Hypoxia Angiotensin II Epinephrine Cytokines Growth factor	CHF Pulmonary hypertension
TNFa (cytokine)	Proinflammatory	Activated macrophages Failing myocardium	Chronic CHF
C Reactive Protein (acute phase protein)		Inflammation	Chronic valvular disease CHF
Adrenomedullin (peptide)	Natriuretic	Increased volume	Congestion Pulmonary hypertension