Biochemical Markers of Cardiac Disease

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OBJECTIVES

1. Know the difference between leakage and functional cardiac biomarkers and be able to identify examples of each.

2. Be familiar with the clinical indications for measurement of cardiac troponins or natriuretic peptides and the clinical significance of elevations in each of these markers.

3. Be aware of caveats for interpretation of troponin and natriuretic peptide levels.

4. Be familiar with the various types of cardiac troponins and natriuretic peptides and know which isoforms are the most clinically useful.

DEFINITIONS

1. A biomarker is defined as any characteristic that may be objectively measured and evaluated as an indicator of normal biologic processes, pathologic processes or pharmacologic intervention. Blood-based biochemical markers may provide information about the presence and severity of disease, and may offer prognostic value.

2. In the past 10-15 years assays for the detection and quantification of circulating markers of cardiomyocyte damage and function have become available. Measurement of cardiac biomarkers may aid in the diagnosis of cardiovascular disease, help to guide therapeutic decisions and serve as prognostic indicators. Biomarkers can be broadly classified as **leakage markers** and **functional markers**.

3. **Leakage markers**: circulating markers of cell death or damage (e.g. ALT as a marker of hepatocellular damage; troponin I as a marker of cardiomyocyte damage)

4. **Functional markers**: indicators of organ function/response to disease (e.g. BUN to assess renal function; NT-proBNP to assess myocardial stretch/dysfunction)

POTENTIAL CLINICAL USE

1. Screening of breeding populations (e.g. HCM, DCM), early disease detection in at-risk populations (e.g. early CHF in CKCS; sub-clinical HCM in cats).

2. Detection of myocarditis, myocardial infarction, pulmonary hypertension, diagnosis of CHF and differentiation of heart failure from primary respiratory disease.

3. Potential to elucidate other diagnostic findings (equivocal systolic function, arrhythmias, Cardiomegaly, etc.), provide prognostic information, and monitor for disease progression over time.

4. May allow for **monitoring effects of cardiotoxic drugs** (e.g. doxorubicin) or cardiac effects of systemic disease (e.g. arrhythmias in gastric dilation/volvulus syndrome).
5. Sensitive indicators of subclinical myocardial injury or active damage. May allow for early intervention prior to acute decompensation and reduced morbidity and mortality.

LEAKAGE MARKERS

1. Circulating indicators of **compromised myocardial cell integrity**

2. Similar to ALT and AST for the liver, intracellular constituents may be released from cardiomyocytes as a result of *cell rupture or loss of membrane integrity*.

    a. May result from volume or pressure overload, hypoxia, ischemia, toxins calcium-handling abnormalities or inflammatory cytokines
    b. At the cellular level these insults may induce oxidative and mechanical stress, inflammation, degeneration, apoptosis, necrosis, fiber slippage and fibrosis.

TROPONINS

Measurement of **cTnI** is a highly sensitive and specific indicator of myocardial **cell necrosis** and has become the gold standard for the detection of myocardial infarction in human medicine, where it has also been used to guide treatment and assess prognosis

1. **Troponin structure and function**

    a. The cardiac troponin (cTn) complex is comprised of three structurally and functionally different protein subunits: **cTnI, cTnT,** and **cTnC.** The complex is located on the thin filament of the contractile apparatus where it plays a crucial role in mediating excitation-contraction coupling.

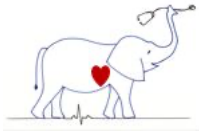
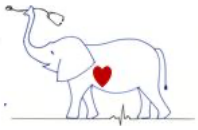
Figures at :
https://www.britannica.com/science/muscle/Actin-myosin-interaction-and-its-regulation
or
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5852618/

- **Troponin-I** 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 is characterized by a 40% dissimilarity from skeletal muscle isoforms.
- **Troponin-T** is responsible for binding the troponin-tropomyosin complex to the actin filament. It is characterized by distinct skeletal and cardiac isoforms
- **Troponin-C** serves to bind intracellular calcium. There is a complete homology between skeletal and cardiac isoforms so measurement of cTnC is non-specific and **not of practical value**.

2. **Troponin release and metabolism**

    a. Injury to the cardiac sarcomere results in release of cTn into the cytoplasm, followed by release into the interstitium. Cardiac lymphatics serve to clear cTn but seepage of cTn into the general circulation is seen when massive release occurs, resulting in detectable cTn serum levels.

    b. Both cTnI and cTnT exist within the cardiomyocyte in a major structurally bound pool and a minor free cytosolic pool. This distribution affects troponin release kinetics; the contents of the cytosolic pool are characterized by an earlier and more transient release than the structural pool.

c. Release of cTn into the circulation occurs secondary to **cardiomyocyte necrosis or compromised membrane integrity**

d. Detectable cTn levels are affected by release from cardiac myocytes, leakage into the general circulation, degradation by serum proteases and clearance by the kidney, liver, and reticuloendothelial system. Impaired renal function in people is associated with increases in cTn levels due to decreased clearance.

3. **Troponin assays**

a. The structure of cardiac troponin isoforms is **closely preserved among species**, allowing for accurate determination of troponin levels in veterinary patients using human troponin immunoassays. Monoclonal antibodies used in 2nd and 3rd generation assays exert no species-specificity or cross-reactivity with skeletal muscle isoforms.

- **Lack of standardization**: Over 16 types of cTnI analyzers are available and absolute troponin levels for a given sample may vary by as much as 100-fold between analyzers due to proprietary differences in the antibodies used. Therefore, cTnI levels obtained from different analyzers cannot be directly compared. Most assays use a two-site sandwich ELISA with two sets of antibodies to capture and label the TnI molecule.

b. Canine and feline reference values have been described for various analyzers and individual reference ranges are needed for each analyzer.

4. **Clinical Applications:**

a. Background troponin levels are *usually* undetectable (0.02-0.03 ng/ml) in healthy dogs and cats but minor elevations in cTnI may result from increasing age, strenuous exercise or non-cardiac disease.

b. Detection of **cTnI** is a more sensitive indicator of myocardial injury than cTnT; cTnI levels become elevated earlier and more readily than those of cTnT.

c. Both cTnI and cTnT are highly specific for myocardial damage and have been shown to correlate with cardiomyocyte necrosis on histopathologic analysis in dogs. They are *not specific*, however, for any particular underlying disease process and circulating troponin levels may be elevated in both primary cardiac and systemic illnesses, including DCM, CHF, trauma, sepsis, uremia and neoplasia. The magnitude of elevation in cTn has been shown to correlate with the degree of myocardial injury in acute situations (GDV, myocardial infarction, myocarditis, sepsis)

d. In one study 47% of dogs with chronic heart disease had elevated cTn levels but no difference was seen between dogs with and without CHF. Initial studies have **variably** demonstrated elevated cTn levels in dogs with pre-clinical DCM thus limiting their usefulness as a screening tool for this disease.

e. In some animals with endocardiosis, or HCM, arteriosclerosis (narrowing of arterioles) may lead to focal myocardial infarction, myocarditis and increased wall stress. Almost all HCM cats have detectable cTn levels regardless of whether CHF is present but **cTn levels are higher in cats with CHF** than in those that are asymptomatic or have had a historic episode of CHF.

f. Troponins may be useful in monitoring the effects of cardiotoxic drugs. **Doxorubicin** chemotherapy has well known **cardiotoxic effects** and in people and early release of cTn following doxorubicin administration may herald the development of impending systolic

dysfunction. A veterinary study showed increased cTnT levels 2-3 weeks after doxorubicin chemotherapy and suggested that serial monitoring of cTn levels after Adriamycin chemotherapy may allow for earlier detection of cardiotoxicity and help to guide therapeutic decisions.

g. Elevated serum concentrations of cardiac troponin I (cTnI) have been demonstrated in dogs with pericardial effusion, and dogs with hemangiosarcoma may have significantly higher cTnI levels than those with idiopathic pericardial effusion.

5. Other leakage markers

a. **Lactate dehydrogenase, myoglobin, and creatine kinase isoenzyme MB (CK-MB)** are no longer recommended for veterinary use due to the higher sensitivity and specificity of cTn testing.

FUNCTIONAL MARKERS OF HEART DISEASE

Circulating markers of CV function may be used to diagnose, further characterize and prognosticate disease and monitor response to therapy.

1. **Natriuretic Peptides**

a. Family of structurally similar genetically distinct proteins including atrial natriuretic peptides (ANP), brain natriuretic peptides (BNP), C-type natriuretic peptides (CNP), dendroaspis natriuretic peptides (DNP), and urodilatin.

b. Regulators of salt and water homeostasis and blood pressure.

2. **Atrial Natriuretic Peptides**

a. ANP is chiefly produced in the atria but expression by ventricular myocardium is increased in disease states.

b. ANP is synthesized as pre-proANP which undergoes cleavage of a signal peptide yielding pro-ANP. ProANP is stored in atrial myocyte granules; it is cleaved into inactive NT-proANP and active C-terminal ANP which are released into blood in response to atrial stretch and dilation. Canine and human ANP are very similar in structure.

Figures at:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4855512/

or

https://www.sciencedirect.com/science/article/pii/S0196978118301104?via%3Dihub

3. Brain Natriuretic Peptides

a. Also produced primarily in the atria with very little synthesis in ventricular myocardium under normal circumstances. However, chronic pressure or volume overload states lead to increased ventricular synthesis and ventricular myocytes become the major source of BNP. Although small amounts of BNP are stored in granules along with ANP, BNP synthesis and excretion are largely controlled at the level of gene expression.

b. Single precursor molecule pre-proBNP processed into active BNP and biologically inactive NT-proBNP. NT-proBNP is characterized by higher serum

concentrations and a longer half-life than BNP and may be a more clinically useful biomarker.

c. Unlike ANP, there is much variability in inter-species length, structure and action of BNP and species-specific analyzers are needed.

4. Physiologic actions of the natriuretic peptides (NP)

a. Released in response to atrial stretch and dilation that accompany congestion or increased ventricular wall stress as may accompany cardiomyopathies.

b. Exert potent natriuresis, diuresis, and balanced vasodilatation.

c . Release of ANP from atrial granules is proportional to the degree of atrial stretch or increased heart rate. ANP levels may increase rapidly in response to changes in posture and volume status and tachycardia; thus ANP is a marker of acute atrial distention.

d. Counteract RAAS and sympathetic nervous system, inhibit vasopressin release, and modulate cardiomyocyte hypertrophy and fibrosis. ANP also increases vascular permeability thus promoting redistribution from the intravascular to extravascular space.

e. As BNP synthesis is controlled at the level of transcription, a longer term stimulus is required for release and BNP is less susceptible to rapid fluctuations in hemodynamic status. Production is increased in response to wall stretch and wall tension and may be increased in the face of ventricular dysfunction and hypertrophy even in the absence of elevated filling pressures.

5. NP assays

a. Commercial assays for the natriuretic peptides are not standardized and inter-assay results may differ markedly.

b. While the antibodies used in ANP assays are universal, BNP assays are highly species-specific and human assays ***cannot*** be used for analysis of BNP in veterinary patients. An assay for canine **NT-proBNP** has recently become commercially available from IDEXX and was officially launched in January of 2008.

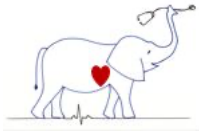
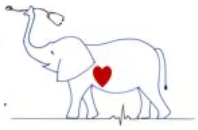
c. Normal dogs and cats have very low levels of circulating ANP and BNP. Reference limits vary according to sample populations and assay used. NP levels may be affected by age, gender and body weight.

6. Clinical applications

a. Increased circulating NP levels are associated with volume overload states (CHF), ventricular hypertrophy, decreased renal clearance, tachycardia, hypoxia, etc.

b. They may have clinical use as non-specific functional markers of heart disease and have been shown to be increased in multiple disease conditions, including chronic valvular disease, DCM, tachycardiomyopathy, aortic stenosis and heartworm disease.

c. Numerous human studies and some canine studies have demonstrated the usefulness of BNP in differentiating cardiac from primary respiratory disease. The stability of NT-

proBNP makes it a more useful clinical biomarker and studies are ongoing to confirm its usefulness in the dog and cat.

- **NT-proBNP** is a sensitive and specific marker for the detection of cardiomyopathy and valvular disease; its levels are correlated with the degree of LV dysfunction, increasing heart size, and worsening CHF class. Measuring NTproBNP can be useful in predicting how close dogs are to developing congestive heart failure and can be used to guide treatment decisions.

d. The atria are the main source of ANP while the ventricles are the main source of BNP (there is some overlap here).

e. Use for screening of asymptomatic patients is not well-established in veterinary medicine. A subset of chronic valvular disease and DCM patients have elevations in NT-proANP; feline cardiomyopathies are associated with pronounced increases in NT-proANP and NT-proBNP; and Dobermans with occult cardiomyopathy characterized by contractile dysfunction have elevated NT-proBNP. The NP may have potential for use as screening tools in at-risk or breeding populations.

7. Endothelin

a. Family of vasoactive peptides, including. ET-1, -2, -3, and -4

b. Large precursor molecule pre-proET cleaved by endopeptidases to form inactive pro-ET, known as big ET. Big ET is converted into active ET by endothelin-converting enzyme (ECE). Primary

c. Wide spectrum of ET-1 biologic actions mediated through 3 receptors: ET_A, ET_B, and ET_C.

- Potent non-selective vasoconstriction with inotropic and mitogenic effects.
- Mediation of adrenergic and RAAS activation crucial role in maintaining vascular tone and blood pressure.

d. ET is not stored in cells and production is regulated at the level of gene expression.

- Stimuli for release include pulsatile vessel stretch, low shear stress, hypoxia, angiotensin II, epinephrine, cytokines and growth factor. Release occurs within minutes of activation.
- Plasma ET-1 level may not reflect ongoing synthesis as the plasma half-life of ET-1 is short (1-4 minutes in humans) and tissue concentrations may be 100-fold those of plasma. Plasma big ET-1 is cleared more gradually and plasma big ET-1 level may be a more accurate indicator of ET-1 synthesis.
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e. Assays:
- RIA or ELISA methods-again assays lack standardization
  - May be increased with CHF and pulmonary hypertension.

8. Cytokines and miscellaneous markers

a. **TNF-α**
- Proinflammatory cytokine produced in activated macrophages and failing myocardium
- Increased in chronic CHF and may be used as an indicator of disease progression.

b. **C-reactive protein (CRP)**
   - Acute phase protein produced by the liver; marker of systemic inflammation.
   - Elevated in dogs with chronic valvular disease, and dogs with CHF

c. **Nitric Oxide (NO)**
   - Potent vasodilator; antagonist of ET and angiotensin II
   - Impaired endothelial release and enhanced myocardial release in CHF may result in endothelial dysfunction (impaired vasodilation), reduced contractile function and cardiomyocyte loss.

d. **Adrenomedullin**
   - Vasodilatory, natriuretic peptide
   - Increased in congestive states and pulmonary hypertension

Cardiac biomarker research is a rapidly evolving field. Biomarkers are being increasingly utilized and several other cardiac markers are under active research in human and veterinary medicine. Measurement of multimarker "panels", as opposed to a single cardiac biomarker will likely be commonplace within the next several years.

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