EDUCATIONAL COMMENTARY – BIOCHEMICAL MARKERS OF CARDIAC FUNCTION

Learning Objectives
Upon completion of this exercise the participant will be able to:

- list and discuss the recommended biomarkers for diagnosis of myocardial infarction and heart failure.
- list and discuss the recommended biomarkers for risk stratification of myocardial infarction and heart failure.
- explain the process for determination of decision cutoff levels for diagnosis of myocardial infarction and heart failure.

An estimated 8 million patients with non-traumatic chest pain present for emergency evaluation annually in the United States. Patients with acute coronary syndrome (ACS), a continuum of disease caused by acute myocardial ischemia, should be rapidly and accurately diagnosed because they have a higher risk for cardiac death or ischemic complications. The diagnosis of ACS is made on the basis of history, physical examination, symptoms, the 12-lead electrocardiogram (ECG) at presentation, and detection of biomarkers of myocardial injury. The results of the ECG are used to divide ACS patients into two major categories: (1) those with new ST-segment elevation on the ECG that is diagnostic of acute ST-elevation myocardial infarction (STEMI) and (2) those who present with ST-segment depression, T-wave changes, or no ECG abnormalities (non-ST elevation ACS, NSTEMI).

The most common cause of ACS is atherosclerotic coronary artery disease with formation of an intracoronary thrombus due to rupture of the atherosclerotic plaque. In approximately 30% of patients with ACS, the thrombus completely occludes the artery resulting in STEMI. Treatment for these patients is typically immediate reperfusion therapy with either fibrinolysis or percutaneous coronary stent intervention.

In the majority of patients with ACS, the vessel is only partially or transiently occluded resulting in either unstable angina or NSTEMI, closely related conditions differing in severity. NSTEMI and unstable angina seem to have the same pathogenesis, but NSTEMI is characterized by ischemia causing severe irreversible myocardial damage, which may be diagnosed by the detection of biomarkers of myocardial injury. Treatment of these patients with fibrinolysis may be harmful. Several biomarkers of myocardial injury have been used historically and have been discussed in a previous educational commentary (See Cardiac Markers Update, API Chemistry Educational Commentary, 2006 1st Test Event).

In addition to determining whether a patient’s chest pain is related to ACS, the initial evaluation of patients should also assess the risk of recurrent cardiac events. This process is often called risk stratification, and the use of cardiac markers for this purpose has evolved in recent years.

Heart Failure
Heart failure is a complex clinical syndrome characterized by an impaired ability of the ventricles to fill with or
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to eject blood. Approximately 2 to 3% of the United States population is affected by heart failure. An increasing number of patients suffer from heart failure because of the aging population and the increased survival rate of patients with myocardial infarction (MI). Myocardial ventricular wall stress stimulates release and cleavage of proBNP into B-type natriuretic peptide (BNP) and the biologically inactive fragment N terminal (NT)-proBNP. B-type natriuretic peptide, an antagonist to the renin-angiotensin-aldosterone system, helps to decrease blood pressure. The use of the hemodynamic stress biomarkers BNP and NT-proBNP for the diagnosis and monitoring of heart failure has been discussed in a previous commentary (BNP and NT-proBNP Update, API Chemistry Educational Commentary, 2006 3rd Test Event). In addition to confirming the presence or absence of heart failure, biomarker monitoring of these patients may help to identify possible underlying causes of heart failure and may provide information for estimation of severity of heart failure and risk stratification for disease progression.

Guidelines
Evidence-based results from several clinical studies have provided the data used by different clinical groups when developing and publishing guidelines for the use of cardiac function biomarkers. In 2007, the National Academy of Clinical Biochemistry (NACB) published Laboratory Medicine Practice Guidelines: Biomarkers of Acute Coronary Syndrome and Heart Failure. These guidelines were developed by a committee of laboratory medicine professionals and cardiology experts and provide analytical and clinical guidance for the measurement and interpretation of cardiac biochemical markers of both ACS and heart failure. Prior to publication, the draft guidelines were placed for comment on the NACB website, presented at conferences, reviewed by representatives from more than 20 stakeholder groups worldwide, and then revised based on the input received. The primary recommendations are presented here, with emphasis on the implications for the laboratory.

Diagnosis and Follow-Up Testing of Myocardial Infarction
One of the primary recommendations is that cardiac troponin (T or I) is the preferred marker for the diagnosis of myocardial infarction (MI) and that when troponin is not available, creatine kinase MB (CK-MB) by mass assay is an acceptable alternative. Thus, the practice of using both assays for the diagnosis of MI is not necessary. Blood for testing should be obtained at hospital presentation followed by serial sampling, typically at 6 to 9 hours. When clinical history is suggestive of ACS myocardial necrosis, a diagnosis of MI is confirmed by the occurrence of either a maximal concentration of cardiac troponin exceeding the 99th percentile of values for a reference control group on at least one occasion during the first 24 hours after the clinical event, or a maximal concentration of CK-MB exceeding the 99th percentile of values for a sex-specific reference control group on two successive samples. Myoglobin testing, the most extensively studied early marker of myocardial necrosis, may be considered for patients who present within 6 hours of the onset of symptoms. Biomarker testing after the diagnosis of MI is valuable for the qualitative estimation of infarction
size and to detect complications such as re-infarction. Testing for these purposes can be done at a reduced frequency of every 6 to 10 hours. If the cardiac troponin concentration is still increased, CK-MB is the preferred marker for detecting re-infarction, but serial measurement of troponin may be used as an alternative.

**Risk Stratification in ACS**
Guidelines on using biomarkers for early risk stratification of patients with suspected ACS are very similar to those for diagnosis of ACS. Integrated assessment should include consideration of symptoms, physical examination findings, and ECG findings in addition to using cardiac troponin as the preferred marker for risk stratification. In these patients, a maximal (peak) concentration exceeding the 99th percentile of values for a reference control group should be considered indicative of increased risk of death and recurrent ischemic events. Times for blood sampling for risk stratification are the same as for diagnosis of ACS (i.e., at hospital presentation and at 6 to 9 hours). In addition to troponin, the use of high-sensitivity C-reactive protein (hs-CRP) (High Sensitivity CRP in Cardiovascular Disease, API Immunology Educational Commentary, 2005 3rd Test Event) and BNP or NT-proBNP may be useful for risk assessment in these patients.

**Analytical Aspects of ACS Biomarkers**
The recommended optimum cutoff decision-limits for myocardial injury for cardiac troponin I (cTnI) and T (cTnT) and CK-MB mass is the 99th percentile of a population of normal, healthy individuals (minimum of 120 for appropriate statistical determination) without a known history of heart disease. For myoglobin, the sex-specific recommended cutoff is the 97.5th percentile of the appropriate reference group. The goal for total imprecision (%CV) is 10% at the 99th percentile reference limit. Studies have demonstrated that this goal is not routinely achieved with the first-generation troponin assays, but the improved second-generation assays have recently demonstrated 10% CVs at the 99th percentile. Achievement of the goal to have all cTn assays attain a 10% CV at the 99th percentile reference limit would reduce the potential for false-positive analytic results caused by imprecision in the low concentration range. The committee recognized that most laboratories do not have the resources to properly determine the recommended 99th percentile cutoff limits and corresponding imprecision, and therefore must rely on external sources for this information. To date very few manufacturers have reported 99th percentile limits, and findings reported in peer-reviewed literature remain the primary source for these limits as well as for the assay imprecision at these limits.

**Diagnosis and Risk Stratification of Heart Failure**
Recommendations for the use of biochemical markers for diagnosis and risk stratification of heart failure are similar to those for ACS if BNP or NT-proBNP testing is substituted for troponin testing. Thus, BNP or NT-proBNP testing is the recommended test for diagnosis of heart failure in both the acute and nonacute setting, and serial measurement of these analytes is also a useful addition to clinical assessment when additional risk stratification is required.
In addition, cardiac troponin testing can help identify patients with heart failure at increased risk even if they do not have ACS. The use of BNP or NT-proBNP to screen for heart failure remains controversial and is not recommended.

**Analytical Aspects of Heart Failure Biomarkers**

The recommended upper reference limits are the 97.5\(^{th}\) percentile of the reference value distribution and should be independently established for both BNP and NT-proBNP based on age, by decade, and by gender. The current medical decision cutoff for heart failure is 100 ng/L for BNP and was determined using the Biosite assay (Biosite Incorporated, San Diego, CA). Harmonization studies should be performed to ensure that this cutoff is appropriate for each laboratory’s population and method of BNP analysis. Harmonization of NT-proBNP assays should not be a problem because Roche Diagnostics (Mannheim, Germany) is the sole source of antibodies and calibrators for these assays. The goal for total imprecision (%CV) for both BNP and NT-proBNP assays is 15% at the 99\(^{th}\) percentile reference limit. Because intraindividual biological variation for both BNP and NT-proBNP can be up to 100%, caution should be exercised in interpreting concentration changes; however, consistent trends should be interpreted as clinically significant.

**Summary and Conclusions**

The recommended biomarkers for the diagnosis of ACS and heart failure are cardiac troponin and BNP / NT-proBNP, respectively. For risk stratification of ACS, troponin, hsCRP, and BNP/NT-proBNP are recommended. Risk stratification of heart failure should include BNP/NT-proBNP and troponin. Because the recommended decision cutoffs are very low (99\(^{th}\) percentile for troponin and 97.5\(^{th}\) percentile for BNP / NT-proBNP) the total imprecision of these assays should be reduced to ensure that the goals (CV less than 10\% for troponin and less than 15\% for BNP/NT-proBNP) are attained. As these assays become more frequently used for risk stratification, laboratory professionals must be vigilant in understanding the analytical parameters of their assays, and thus, maintaining the accuracy, precision, and sensitivity required to distinguish between small changes in the concentration of these cardiac biomarkers.

**Suggested Reading**


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