Review Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

CLINICAL SIGNIFICANCE AND EFFECTIVENESS OF VARIOUS SERUM BIOMARKERS IN DIAGNOSIS OF MYOCARDIAL INFARCTION

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Received for publication: February 28, 2013; **Revised:** April 09, 2013; **Accepted:** May 21, 2013

Abstract: Myocardial infarction (MI) causes significant mortality and morbidity. Timely diagnosis allows clinicians to risk stratify their patients and select appropriate treatment. Biochemical markers play a pivotal role in the diagnosis and management of patients with acute myocardial infarction. The older biomarkers like aspartate transaminase, creatine kinase, lactate dehydrogenase has lost their utility due to lack of specificity and limited sensitivity. This paper reviews the current contribution of the biochemical marker determination to clinical cardiology and discusses some important developments in this field. Despite the success of cardiac troponins which are gold standard, there is still a need for the development of early markers that can reliably rule out acute myocardial infarction from the emergency room at presentation and also detect myocardial ischaemia in the absence of irreversible myocyte injury. Since no single biomarker fulfils the criteria of ideal biomarker, the National Academy of Clinical Biochemistry (NACB) proposes the use of two biomarkers for the diagnosis of acute myocardial infarction: early marker – myoglobin and a definitive marker – cardiac troponins. Among the new biomarkers, heart type fatty acid binding protein, glycogen phosphorylase isoenzyme BB, ischaemia modified albumin seem to be promising.

Key words: Biomarkers, Myocardial Infarction, Cardiac Troponins, CK-MB

INTRODUCTION

The term acute myocardial infarction (MI) should be used when there is evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischaemia. Under these conditions, any one of the following criteria meets the diagnosis of MI:

- Detection of a rise and/ or fall of cardiac biomarker values (preferably cardiac troponin cTn) with at least one value above the 99th percentile upper reference limit (URL) and with at least one of the following:
- Symptoms of ischaemia
- New or presumed new significant ST segment T wave (ST-T) changes or new left bundle branch block (LBBB) [1].

The rationale of using the measurement of a protein in blood to detect injury to cells is straightforward. The myocyte is the major cell in the heart, and the heart's purpose is to pump blood. Because myocytes essentially cannot be regenerated, if heart cells die, then cardiac function has a high probability of being impaired. When the cell dies, the proteins inside the cell will be released, with proteins in the cytoplasm leaving the cell more rapidly than ones in membranes or fixed cell elements. The most sensitive markers should be those in highest abundance in the cell, and because the major function of the heart is contraction, the proteins involved in contraction and producing the energy to support it should be good candidates for biomarkers of cardiac injury which could be detected in blood [2].

Definition of biomarker:

The National Institutes of Health's Biomarkers and Surrogate Endpoint Working Group defines a biological biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [3].

For years, the gold standard for diagnosis of myocardial necrosis was the cardiac-specific isoenzyme of creatine kinase (CK-MB). The National Academy of Clinical Biochemistry (NACB) committee recommends that cardiac troponin T or I become the new standard for diagnosis of MI and detection of myocardial cell damage. In the past, measurement of non specific markers such as aspartate transaminase, lactate dehydrogenase and total creatine kinase were performed. They were performed by electrophoretic technique. Then during 1990's, the electrophoretic methods were replaced by CK-MB mass assays using automated immunodiagnostic instruments [4].

According to NACB (National Academy of Clinical Biochemistry) recommendations, two serum cardiac

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markers need to be determined for routine diagnosis of acute myocardial infarction, i.e one showing early elevation in serum (up to 6 hours after chest pain), and the other late marker that is elevated six to nine hours after chest pain has high sensitivity and specificity for detection of myocardial injury, and remain elevated for several days of the symptom onset.

To meet the clinical requirements, a marker ideally should be:

1. Highly cardiac specific to allow reliable diagnosis of myocardial damage in the presence of skeletal muscle injury.
2. Highly sensitive, the marker should detect even small myocardial damage.
3. Suitable for early as well as late diagnosis.
4. Measurable with a rapid, easy to perform and cost effective assay [5].

Biochemical markers of myocardial infarction in serum/ plasma:

1. Lactate dehydrogenase
2. Aspartate transaminase
3. Creatine kinase - MB
4. Cardiac troponins
5. Myoglobin
6. Myeloperoxidase
7. Heart type fatty acid binding protein
8. B-type natriuretic peptide
9. High sensitive C-reactive proten
10. Copeptin
11. Ischaemia modified albumin
12. Glycogen phosphorylase isoenzyme BB

Lactate dehydrogenase (LDH):

In addition to heart muscle, LDH occurs in many other parts of the body, including the kidneys, red blood cells, brain, stomach, and skeletal muscle. Five LDH isoenzymes are known, composed of four subunit peptides designated M and H. The LDH1 isoenzyme is found in highest concentration in the heart, kidney, and red blood cells. The LDH5 isoenzyme is found in the highest concentration in the liver and skeletal muscle [6]. The LDH2, LDH3, and LDH4 are found in the heart, kidney, red blood cells, and several other tissues. Of the five LDH isoenzymes, LDH1 and LDH2, are useful in the diagnosis of myocardial ischemia.

Levels of LDH start to increase 24 to 48 hours after myocardial infarction, peaks in 3 to 6 days, and return to normal in 8 to 14 days [7]. Levels of LDH1 are elevated 10-12 hours after AMI, peaks in 2 – 3 days and returns to normal in approximately 7 to 10 days [8, 6]. Thus, LDH serves as a late marker of AMI. The amount of LDH2 in the blood is usually higher than that of LDH1, (or LDH1/LDH2 <1.0); however, patients with AMI have more LDH1 than LDH2, (or LDH1/LDH2 > 1.0). This "flipped ratio" usually returns to normal in 7 to 10 days. An elevated level of LDH1 with a flipped ratio has a sensitivity and specificity of approximately 75% to 90% for detection of AMI [9].

Aspartate transaminase:

Aspartate Aminotransferase (AST) is principally found in liver, myocardium, skeletal muscle and kidney. AST rises and falls after acute myocardial infarction (AMI) in a pattern similar to that of CK - slightly later and slightly less when activities are expressed as multiples of the upper reference limit. It can be elevated in patients with skeletal muscle disease, pulmonary emboli, hepatic disease and also by intramuscular injections [10]. Serum activity of AST is noticeably increased after about 6-12 hours, peaks between 1-2 days, and returns to normal by the 3rd to 5th day.

Creatine kinase –MB:

Creatine kinase – MB (CK-MB) is found almost entirely in myocardial tissue and elevation of this isoenzyme became the gold standard marker for MI. CK-MB level typically rises 6 to 10 hours after the onset of chest pain in MI patients, peaks at 12 to 24 hours, and returns to baseline levels within 72 hours. The magnitude and temporal course of CK-MB elevation and decline have been shown to correlate strongly with infarct size [11]. Since CK-MB is found in both cardiac muscle and skeletal muscle, damage to either may increase the serum level. A measurement known as the Relative Index (RI) is used to distinguish cardiac from skeletal muscle damage. The ratio is: (CK-MB/Total CK) x 100. If the RI is ≥ 5%, this is consistent with myocardial damage.

In electrical shock or convulsions, the total CK is quite increased and CK-MB is also high, but the RI remains normal. Marked elevation of cardiac markers, especially total CK and CK-MB occurs following use of "clot busting therapy" such as tissue plasminogen activator (TPA or streptokinase), resulting in a "washout" phenomenon. Values of total CK and CK-MB may achieve levels of 10 to 20 x upper limit of normal. These values must not be confused with a massive AMI [12].

Cardiac troponins:

Troponin (Tn) is a complex of three proteins on the thin filaments of skeletal and cardiac muscle fibres. During muscle contraction the troponin complex regulates the interaction between the thick and thin filaments. This complex consists of troponin T (TnT; Tropomyosin binding), troponin I (TnI, Inhibitory component) and troponin C (TnC, calcium binding component). Troponin C is identical in skeletal and cardiac muscle but the amino acid sequences of troponin T and troponin I found in cardiac muscle are different from that of the troponins in skeletal muscle.

These isoforms of cardiac troponins, cTnT and cTnI, are very specific to cardiac muscle and their presence in blood indicates cardiac tissue necrosis. Also, cardiac troponins have been established as sensitive and specific markers of minor myocardial lesions in patients with acute coronary syndrome [13, 14, 15]. Because of this specificity, cardiac troponin T or I is now the preferred cardiac marker. Both troponins are considered to be acceptable [16]. Cardiac troponins T and I begin to rise 4-8 hours after myocardial damage, peak at approximately 12 - 24 hours, and remain elevated for up to 10 days.

An increased circulating cardiac troponin concentration indicates myocardial injury and aids in the diagnosis of acute myocardial infarction (AMI) [17, 18]. The risk of both short and long-term cardiac events and mortality is related strongly and directly to increased cardiac troponin concentrations in patients who present with symptoms of acute coronary syndrome (ACS) [19].

The prognostic information obtained from the measurement of cardiac troponin I or T (cTnI or cTnT) has been shown to be independent of clinical risk factors, such as age, electrocardiogram (ECG) results, renal disease, and diabetes mellitus [20]. International associations of cardiology, laboratory medicine, epidemiology, and emergency medicine have all issued guidelines that have designated cardiac troponin as the preferred biomarker, both for aiding in MI diagnosis and for risk stratification in patients presenting with suspected ACS [21].

Myoglobin:

Myoglobin is the only haem protein in cardiac myocytes, usually released more rapidly into blood than any other cardiac marker because of its small size. Myoglobin is low molecular weight protein that binds oxygen in muscle and damage to any muscle tissue will result in elevation of myoglobin in blood [22].

Myoglobin is detectable in blood as early as 2 – 3 hrs after onset, its concentration appears to peak quickly, reaching the maximum level between 6 and 12 hrs after the onset of symptoms, then normals over the next 24 hrs. Myoglobin is not cardiac specific and patients with renal failure, skeletal muscle injury or trauma can have abnormal concentration in the absence of AMI. But despite this limitation, myoglobin has potential utility as test excluding early AMI in patients presenting to the emergency department with chest pain [23].

Myeloperoxidase:

Myeloperoxidase is a neutrophil and monocyte enzyme that amplifies the reactivity of hydrogen peroxide through generation of hypochlorous acid, free radicals and reactive nitrogen species [24]. Myeloperoxidase and products of protein oxidation by hypochlorous acid have been detected in atheromatous lesions [25, 26, 27]. Myeloperoxidase is a marker of plaque instability and therefore presents as a potential strong prognostic marker of myocardial infarction in near future. Myeloperoxidase is lowest in patients with stable coronary artery disease, higher in patients with unstable angina and highest in patients with acute myocardial infarction [28].

Heart type fatty acid binding protein:

It is a low molecular weight (15 KD) cytoplasmic protein present in myocardium and is released into the circulation following myocardial injury. Its plasma kinetics closely resembles those of myoglobin but it is more cardiospecific than myoglobin. It was found to be elevated within 3 hours after AMI and return to normal levels within 12-24 hours. Hence it is considered as a sensitive and specific marker of early detection of myocardial injury as compared to CK-MB and myoglobin [29, 30].

B-type natriuretic peptide:

B- type natriuretic peptide is a neurohormone produced in the ventricular myocardium in response to dilation and pressure overload, and its plasma concentration correlates with the magnitude of pressure and/or volume overload [31, 32]. Both the active form BNP and the inactivated N--Terminal peptide "NT--proBNP" can be measured as markers of hemodynamic stress. While investigations have shown that elevated BNP and NTproBNP levels are predictive of death and heart failure, they are not useful as indicators of new or recurrent AMI [33, 34]. More research is being conducted to establish the use of these biomarkers for selecting treatment of acute coronary syndromes [35].

High sensitive C-reactive protein:

C-reactive protein is an acute phase protein originally named by Tillet and Francis in 1930 [36]. It is produced by the liver in response to inflammation and infection. It increases rapidly following many disease conditions such as infections, trauma, and surgery

An elevated C-reactive protein measured in seemingly healthy adults was associated with increased cardiovascular risk [37]. CRP itself mediates atherothrombosis [38, 39, 40]. This is supported by a fairly large body of evidence. Newer, higher sensitivity assays of CRP that detect lower levels of CRP (<5 mg/L) risk stratify patients into low, intermediate and high risk, with intermediate and high risk individuals benefiting from aggressive therapy [41]. While the benefits of HsCRP testing in a primary setting to screen for ischaemic heart disease is very clear, its use post ACS or MI is less clear. CRP is elevated post-acute

coronary syndrome almost exclusively in the setting of myocardial necrosis indicating the level of myocardial inflammation.

Copeptin:

Copeptin is the C--terminal fragment of the vasopressin precursor hormone which is released in response to low blood pressure. Also, the measurement of copeptin has been shown to have very strong negative predictive value, along with troponin, for AMI [42]. Additionally, copeptin levels are elevated early after AMI and are detectable in patients who present soon after symptom onset while troponin is still negative [43].

Ischaemia modified albumin:

Studies have shown that the structure of serum albumin changes when ischemia develops in the body. Normal human albumin has the N terminal region, which detoxifies free oxygen radicals. The N region is also the area to which such transition metals as cobalt, copper and nickel are bound. In the ischemia condition, the N region is damaged by oxygen radicals. Albumin's capacity to bind metals such as nickel, cobalt and copper is diminished [44, 45]. The resultant albumin as such is ischaemia modified albumin. The type of the radical that affects the N terminal region most is the hydroxyl radical. Free radical binding capacity of ischaemia modified albumin is very low. Elevation of ischaemia modified albumin is directly associated with free radicals that form during ischemia [46]. ischaemia modified albumin is also one of the markers of oxidative stress [47, 48].

The most important characteristic that differentiates ischaemia modified albumin from other cardiac ischemia markers is that it increases in the early phase particularly. It elevates in just minutes, peaks within 2 to 4 hours and returns to normal in 6 to 12 hours [49, 50]. Non-existence of myocardial ischemia is considered to be confirmed in 90-95% of the cases if ischaemia modified albumin levels are normal in the presence of a normal ECG and normal troponin levels [51]. Since ischaemia modified albumin is an early marker, measurement of ischaemia modified albumin levels may contribute to patient follow up and initiation of treatment at early stages.

Glycogen phosphorylase isoenzyme BB:

Glycogen phosphorylase (GP) is bound to glycogen in the sarcoplasmic reticulum and catalyzes the first step of glycogenolysis after activation, which involves the separation of glucose-1-phosphate from glycogen [52]. Three GP isoenzymes are present in human tissue. These are named according to the tissue of their initial description: GPLL (liver), GPMM (muscle) and GPBB (brain) [53].

Skeletal muscles contain GPMM exclusively. GPLL is present in all tissues except the brain, heart and skeletal muscles. In addition to its occurrence in brain, GPBB is also found in high concentrations in heart muscle (next to GPMM) [54]. During myocardial ischemia, activation of GPBB results in an increase in glycogen degradation. Thus, GPBB is released from glycogen and then enters the bloodstream, which is believed to occur via the T-tubulus system [55]. Initial research has shown higher sensitivity of GPBB within the first 4 h following chest-pain onset in comparison to other cardiac markers. In addition, GPBB seems to indicate necrotic cell damage and, in particular, ischemic processes, e.g., as observed with unstable angina pectoris (UAP) [56].

Glycogen phosphorylase-BB appears to be released into the circulation 2 - 4 hours after myocardial injury, [57] returning to normal within 36 hours of damage occurring. It has been reported to be a useful marker of myocardial damage following bypass surgery [58].

CONCLUSION

The use of cardiac biomarkers has greatly improved the diagnostics of myocardial infarction. Currently the best marker available in clinical practice is cardiac troponin. Although there is evidence that combining biomarkers may increase the accuracy of the tests, the best combinations for diagnosis or prognosis need to be defined. Cardiac marker testing in the clinical laboratory continues to evolve based on the availability of improved technologies and the results of clinical trials among patients with acute myocardial infarction.

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Source of support: Nil
Conflict of interest: None Declared