Mini-review

LABORATORY DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

A. Ruseva*

Central Clinical Laboratory, University Hospital, Pleven, Bulgaria

ABSTRACT

The early recognition of cardiac ischemia and accurate placement of the patient in the risk spectrum of the acute coronary syndrome are critical to the effective management of patients with acute myocardial infarction (AMI). Apart from clinical history, physical examination and accurate ECG interpretations, cardiac biomarkers are equally valuable in the initial evaluation of patients with non-traumatic chest pain. Previously the diagnosis of an AMI was based on World Health Organisation (WHO) criteria which defined MI as the presence of two out of three characteristics comprising: symptoms of acute ischemia (chest pain), development of Q waves in ECG and elevated activities of traditional serum enzymes comprising: total CK, CK-MB, ASAT and LDH. An ideal biomarker should have the following characteristics: relatively high concentration within cardiac tissue, have no significant tissue sources other than the heart, have high clinical sensitivity and specificity, be detectable in the blood early after the onset of chest pain, have elevated blood levels for several days after the onset of symptoms, and have an assay with a quick turnaround time. Since no single biomarker fulfils all of these criteria, the NACB proposes the use of two biomarkers for the diagnosis of AMI: an early marker – myoglobin and a definitive marker- cardiac troponins. When cardiac troponin is not available, the next best alternative is CK-MB (measured by mass assay).

Key Words: AMI, laboratory diagnosis, troponin, myoglobin, CK-MB

When a patient presents with chest pain in the emergency department, physicians must consider a continuum of acute coronary syndromes which could include the following: non-cardiac chest pain, unstable angina in which oxygen deprivation occurs without permanent damage to heart muscle and heart attack or myocardial infarction (MI) with permanent heart muscle damage. If patients with chest pain are not properly evaluated, then some patients without an acute coronary event will be inappropriately admitted, while patients experiencing an AMI may be discharged[25]. Critical to the effective management of these patients are the early recognition of a cardiac ischemia event and the proper placement of the patient in the risk spectrum of the acute coronary syndrome [20]. The objectives of the initial evaluation of patients with non-traumatic chest pain are twofold:

1. To assess the probability that the patient's symptoms are related to acute coronary ischemia.
2. To assess the patient's risk of recurrent cardiac events, including death and recurrent ischemia [5].

Diagnosis of an AMI in the past, during the early 1990s, utilised the World Health Organisation (WHO) criteria defining MI as the presence of two out of three characteristics:

- symptoms of acute ischemia (chest pain);
- development of Q waves in ECG;
- elevation of traditional enzyme activities in serum: total CK, CK-MB, ASAT and LDH [4, 20, 23, 24]

Creatine kinase (CK) emerged as the primary indicator of MI. Total CK starts to rise within 3 to 8 hours after MI, peaks at 10 – 24 hours and returns to normal by 3 – 4 days. It can be markedly elevated with skeletal muscle trauma or brain injury. Other skeletal muscle diseases including dystrophy, myopathy and myositis show increase. Electrical cardio-version shows an increase as does cardiac catheterisation without myocardial damage.

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*Correspondence to: Adelaida Ruseva, MD.
Central Clinical Laboratory, University Hospital,
8-A G. Kotchev Str., 5800 Pleven; E-mail:
adi_ruseva@dir.bg

Total CK is increased in hypothyroidism, stroke, surgery and in patients with convulsions who have skeletal muscle damage. Therefore total CK is not specific for MI.

Three isoenzymes in blood comprise the total fraction of these: CK-MM (CK-1) (Skeletal Muscle) > 95% of total, CK- MB (CK-2) (Myocardial) <5% of total, CK-BB (CK-3) (Brain) 0% of total. CK-MB (CK-2) is found almost entirely in myocardial tissue and elevations 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. [8, 22] 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.

The Lactate Dehydrogenase (LDH) is an enzyme that has five isoenzymes: LDH 1-5. LDH-1 and LDH-2 occur in high concentration in myocardium and in red cells. Ordinarily the concentration of LDH-1 is less than the concentration of LDH-2. The normal ratio of LDH-1 to LDH-2 is <0.7. In myocardial infarction, the ratio increases to >1 and is rarely greater than 1.3. LDH-1 to LDH-2 rise above base line at around 10 hours following myocardial infarction, peak at about 24 to 48 hours and stay elevated in blood for up to 14 days post MI [3, 9, 17].

The Aspartate Aminotransferase (AST) is principally found in liver, myocardium, skeletal muscle and kidney. AST rises and falls after 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. However, in the patient with a minor infarction or a non-Q-wave infarction there may be no change in the AST values [9].

According to the National Academy of Clinical Biochemistry (NACB), do to low specificity and the availability of more specific alternative biomarkers of necrosis, total CK, Lactate Dehydrogenase (LDH) and Aspartate Aminotransferase (AST) should no longer be used for the diagnosis of MI [18].

So, the imperfect sensitivity and specificity of the traditional enzymatic markers for the detection of myocardial injury are well known. The ECG shows in approximately 60% of patients ST segment change within seconds of the ischemic evidence. However, the ECG can be inconclusive in the remaining 40% of cases, therefore, showing a globally low sensitivity. Chest pain is an unreliable indicator: up to 33% of patients with AMI may have no chest pain and are clinically silent. On the other side, many people experience chest pain, resulting from plaque rupture and subsequent deposit of platelets but do not have an AMI. This occlusion may not be large enough to cause an acute infarct but it may cause minor myocardial damage with a result – leak of Cardiac Troponins from the damaged myocytes. They can be detected and measured long before the development of traditional AMI. It was thus necessary to establish a new diagnostic group with troponin-positive patients who did not meet the WHO criteria for AMI. This new category became known as 'acute coronary syndrome' or ACS. It includes AMI as the most serious form of ACS. The new ACS model required new diagnostic criteria to classify AMI and the other ACS stages. In 1999 the U.S. National Academy of Clinical Biochemistry published its guidelines for cardiac marker testing. This guideline specified that the cardiac troponins are the most specific and sensitive available biochemical cardiac markers. While redefining myocardial infarction, recommendations by the Joint European Society of Cardiology and American College of Cardiology (ESC/ACC) Committee in 2000 stated that ECG was no longer sufficient to diagnose AMI. Final diagnose of ACS, according to this committee, should depend on cardiac biomarkers, especially cardiac troponins [4, 20].

Troponin 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. [7, 10, 15, 27] Because of this specificity, cardiac troponin T or I is now the preferred cardiac marker. Both troponins are considered to be acceptable [4, 13, 24].

Troponin T is a cardio-specific polypeptide mostly bound to contractile elements of myocardial cells, but with small amounts also present free in the cytoplasm. Cytosolic cardiac troponin T is released within the first few hours after infarction. Release of myofibrillar cTnT occurs more slowly, over a period of days. This biphasic release results in an early rise in serum levels (3-4 hours after the infarct) which is sustained for 10 days or more. This makes it a very useful marker. Minor elevations occur in unstable angina.

Three distinct tissue-specific isoforms of Troponin I have been identified: two in skeletal muscle and one in cardiac muscle. Although the molecular weight of the two skeletal TnI isoforms is approximately the same (19.8 kDa), the cardiac isoform of TnI (cTnI) has an additional sequence of 31 amino acids at the N-terminus resulting in a molecular weight of 24 kDa. cTnI has never been isolated from skeletal muscle. Within the heart, cTnI appears to be uniformly distributed throughout the atria and ventricles. This absolute specificity of cTnI for cardiac tissue makes it an ideal biomarker of myocardial injury.

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. It should be remembered that cardiac troponins reflect myocardial damage but do not indicate its mechanism. Thus, an elevated value in the absence of clinical evidence of ischemic heart disease should prompt a search for situations in which various degrees of myocardial injury may be present. Elevation of cardiac troponins without ischemic heart disease can be observed in [20]:

- Acute rheumatic fever;
- Amyloidosis;
- Cardiac trauma (including contusion, cardioversion, cardiac surgery);
- Cardiotoxicity from cancer therapy;
- Chronic renal failure;
- Congestive heart failure;
- Hypertension;
- Myocarditis;
- Postoperative noncardiac surgery;
- Pulmonary embolism;
- Sepsis

One of the most important problems in the practical use of cardiac-specific troponins is the right definition of decision limits. The basic question is: 'how much necrosis is needed to make the diagnosis of AMI?' In the purest physiologic sense, the answer is that any detectable necrosis is an AMI. Consequently, even small elevations of specific markers of myocardial damage, such as cardiac troponins, should be acknowledged as indicative of significant myocardial injury. From a clinical perspective, there is clear evidence that any amount of detectable cardiac troponin release is associated with an increased risk of new adverse cardiac events. Currently available data demonstrate no threshold below which elevations of troponin are harmless and without negative implications for prognosis. There are studies which confirmed that optimal risk stratification in patients with acute coronary syndrome can be achieved with use of a cut-off concentration around the detection limit instead of the manufacturer's suggested higher cut-off value. Pragmatically, the use of this approach as a diagnostic criterion for AMI will lead to an increase in the numbers of infarct patients in the acute coronary syndrome population from 15 – 30%. On the other hand, increasing diagnostic sensitivity for AMI can have a positive impact on society, resulting in more cases being identified, thereby allowing appropriate secondary prevention and hopefully reducing health care costs in the future. In a study, patients who had an AMI diagnosis made solely on the basis of a positive troponin value experienced a three-fold increase in short-term mortality compared with the normal troponin group [20].

The document published in 2000 by the European Society of Cardiology and the American College of Cardiology is the

appropriate next step, making specific new recommendation on the use of biomarkers for the detection of myocardial necrosis. In particular, the document considers as the best biochemical indicator for detecting myocardial necrosis a concentration of cardiac troponin exceeding the decision limit on at least one occasion during the first 24 hours after the onset of clinical event. The use of CK-MB, measured by mass assay, is still considered as an acceptable alternative only if cardiac troponin assays are not available. In the light of the lower tissue-specificity compared to troponin, it is recommended that in most situations two consecutive measurements of CK-MB above the decision-limit are required to be considered sufficient biochemical evidence of myocardial necrosis. The redefined criterion used to classify acute coronary syndrome patients with ischemic symptoms as AMI patients is, therefore, heavily predicted on an increased cardiac troponin concentration in blood [18, 20, 23, 26].

The ideal biomarker for detecting myocardial injury needs to be in relatively high concentration within cardiac tissue, have no significant tissue sources other than the heart, have high clinical sensitivity (few false-negatives) and specificity (few false-positives), be detectable in the blood early after the onset of chest pain, have elevated blood levels for several days after the onset of symptoms, and have an assay with a quick turnaround time (such as with an automated analyzer). The National Academy of Clinical Biochemistry (NACB), in conjunction with leading researchers in the field of cardiac biomarkers, have proposed Standards of Laboratory Practices for the use of cardiac markers in coronary artery diseases. Since no single biomarker fulfils all of the criteria of an ideal biomarker, the NACB proposes the use of two biomarkers for the diagnosis of AMI: an early marker and a definitive marker [20, 24].

Blood levels of the early marker must be consistently elevated within the first 6 hours after the onset of symptoms. The NACB proposes myoglobin as the ideal early marker.

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. Myoglobin may rise within the first 1

to 3 hours following MI, peaks at 6 – 9 hours and returns to normal within the first 24 hours since it is rapidly excreted into the urine. It is non-specific for myocardial damage, since both myocardium and skeletal muscle contain high quantities of myoglobin. That is why it should be used as a cardiac marker only in conjunction with specific cardiac markers such as Troponin and/or CK-MB. It has high sensitivity in the uncomplicated patients with chest pain and AMI. [3, 13] Thus, its presence is not diagnostic of AMI, but it still has utility in the early triage of chest pain patients. Myoglobin has potential utility as test for excluding early AMI in patients presenting with chest pain at the emergency department. The negative predictive value of this marker is virtually 100% [6, 19, 20].

The definitive marker must be detectable in the blood within 6 to 9 hours after the onset of symptoms, have high sensitivity and specificity for myocardial injury, and blood levels must remain elevated for several days. The NACB proposes cTnI or cTnT as the ideal markers for a definitive AMI diagnosis.

When cardiac troponin is not available, the next best alternative is CK-MB (measured by mass assay). Testing for cardiac troponin is associated with fewer false positive results in the setting of concomitant skeletal muscle injury, e.g. after trauma or surgery, and also provides superior discrimination of myocardial injury when the concentration of CK-MB is normal or minimally increased [1, 2, 14, 21]. Moreover, the association between an increased concentration of cardiac troponin and a higher risk of recurrent cardiac events in patients with normal serum levels of CK-MB and suspected acute coronary syndrome has confirmed the clinical relevance of detecting circulating troponin in patients previously classified with unstable angina [11, 12, 18, 24].

Some authors have shown data to the effect that a three-test panel, Myoglobin-CKMB-cTn, performed better than the two-panel, Myoglobin-cTn, in the discrimination among chest pain patients without ST-segment elevation who were likely to have an MI or die within the 30-day period following testing. This finding suggested that CK-MB may provide some additional useful information in identifying such patients. CK-MB retains its important historical role as a useful indicator of reinfarction and MI extension, which may occur in patients with an acute coronary syndrome [26].

Figure 1 Plot of cardiac marker levels vs time after onset of symptoms
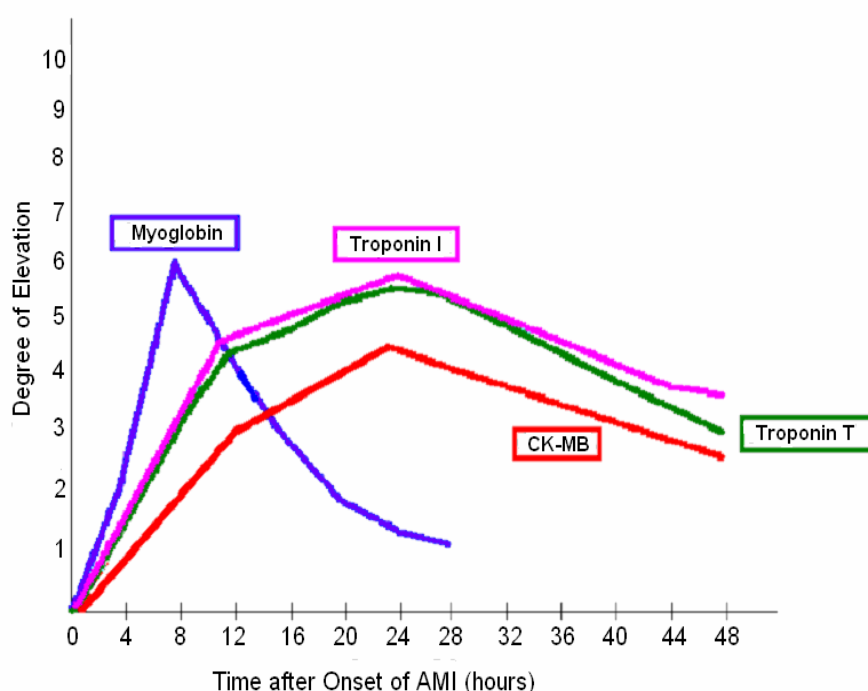
The specimen of choice for all of these cardiac markers is serum. Specimen type and handling and test methodology affect measurements of cardiac markers. Extended storage (days) at room temperature causes a deterioration of CK-MB, but the greater deterioration is seen with electrophoretic methods than with immunoassays. Heparinised plasma is generally acceptable for CK and CK-MB analysis but other anticoagulants may interfere with CK activity. Initially, heparinised plasma was an acceptable specimen for troponin analysis, but subsequent studies have shown decreased troponin levels in plasma and poor correlation between serum and plasma troponin levels. Manufacturers of both troponin T and I assays no longer recommend the use of heparin plasma tubes. CK-MB and troponin are affected by any degree of haemolysis, turbidity, unconjugated hyperbilirubinaemia and conjugated hyperbilirubinaemia, while myoglobin can be affected only by severe unconjugated hyperbilirubinaemia and conjugated hyperbilirubinaemia in the samples with concentration higher than reference range, resulting in concentration level lower than baseline [16].

For detection of AMI by enzyme or protein markers, the following sampling frequency is recommended:

Marker	Admission	2 – 4 h	6 – 9 h	12 – 24 h

| *Early(<6h) | X | X | X | (X) |
| **Late(>6h) | X | X | X | X |

(X)   indicates optional determination
*   indicates marker positive in serum < 6 h after AMI
**   indicates marker positive in serum > 6 h after AMI
Note: Troponins both early/late markers [3]

It is clear that much has been accomplished and much is still left to be done in this field. It is hoped that coming years will be as fruitful as the previous in evaluating new cardiac markers (fatty acid binding protein [FABP], ischemia-modified albumin [IMA], lipoprotein (a) [Lp(a)], and remnant lipoprotein-cholesterol [RLP-C]) and monitoring strategies that improve the diagnosis of AMI.

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