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Editorial

Is there a continual role for serum creatine kinase and myoglobin testing in the era of high sensitivity troponin assays?

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Introduction

Published guidelines from the Joint Global Task Force on the Redefinition of Myocardial Infarction (MI) published in 2000[1] have recommended that cardiac troponin is the "preferred" serum biomarker of diagnosis for MI. The first commercial troponin T assay (cTnT) was approved by the US FDA in 1994. One year later, the first cardiac troponin I (cTnI) assay was cleared. Over the ensuing years, there have been dozens of commercial assays that were made available, with numerous improvements particularly with precision and analytic sensitivity. In the year 2012, The Global Task Force further opines that "if assays for cardiac troponin are not available, the best alternative is creatine kinase-MB (CK-MB) (measured by mass assay)"[2]. Given that the cost of troponin assays is higher than for CK-MB, this recommendation was included to address the needs of countries that are resource limited. Myoglobin testing, thought to be an earlier marker, was not mentioned in the latest Global Task Force document.

For countries that are able to offer cardiac troponin testing, the need to continue to offer testing for CK-MB and myoglobin can be debated. In a study of antiquated tests, a survey of subscriptions to proficiency testing survey was made to track the utilization of CK-MB assays over time.[3] In 1993, the year before the introduction of troponin, there were roughly 3000 laboratories who were testing CK-MB. This increased to a peak of 3500 laboratories in 1999. Over the next 10 years, there was a 22% decline of laboratories with CK-MB testing to around 2700. Today, 8 years later, the number of subscriptions has surprisingly remained steady at around 2700. During this same time interval, the number of laboratories performing troponin testing has exceeded 4000, while myoglobin is only used in a minority of laboratories (only 800 subscriptions in 2017). Despite the predictions that CK-MB will become irrelevant with the advent of assays for cardiac troponin, testing continues to occur at a large number of hospitals and medical centers. This editorial explores the possible reasons for the continued interest and use of CK-MB with predictions and recommendations regarding the future of CK-MB.

Increasing sensitivity of troponin assays reduces clinical specificity

The interest in troponin assays stems from the observation that this biomarker is more analytically sensitive and specific than CK-MB or myoglobin. The increased sensitivity is predicated on the fact that the amount of troponin present in the myocardium is 5–10-fold higher than CK-MB[4] and myoglobin.[5] Therefore, there is more troponin protein release per gram of infarcted tissue.

The improved analytical specificity of troponin is predicated on the fact that CK-MB is found in both striated skeletal muscles and the myocardium. Therefore increased concentrations are observed following damage to both tissues. One means to differentiate the causes of CK-MB increases is the calculation of the "relative index", i.e., the ratio of CK-MB to total CK. Since the myocardial tissue content of CK-MB is higher in the heart, a disproportionate increase relative to total CK is indicative of myocardial injury, while a proportionate increase in both produces a normal relative index and is indicative of skeletal muscle injury as the bulk of CK in blood comes from skeletal muscle turnover. Unfortunately, the CK-MB relative index cannot detect myocardial injury when there is involvement in both the heart and skeletal muscle. A similar

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situation occurs for myoglobin, where the same protein is found in both the heart and skeletal muscle. Non-specificity also plaques other novel early acute myocardial infarction (AMI) biomarkers such as heart-type fatty acid binding protein and copeptin.[6] Cardiac troponin isoforms are structurally distinct from the myocardial forms. Because laboratory assays can be created that detect only the cardiac isoform, the troponin assay is more specific than CK-MB. Increased tissue specificity also enables an increase in analytical sensitivity. Due to the skeletal muscle component, blood of healthy subjects has a considerably baseline concentrations of CK-MB and myoglobin. An increase in serum concentrations following myocardial injury can only be detected when it significantly exceeds the baseline level. Very little troponin is found in health subjects, there must be less protein released before it can be detected above the noise (biological variation of the marker).

The increased analytical sensitivity of troponin has led to decreased *clinical* specificity for AMI diagnosis. In years past, cutoff concentrations for CK-MB and myoglobin were optimized from receiver operating characteristic (ROC) curve analysis to separate AMI from unstable angina. It was correct to label these as "AMI markers". Years later, unstable angina was recognized as part of the spectrum of acute coronary syndromes. As a consequence, the cutoff concentrations for troponin were lowered to detect this clinical entity. Today, cutoff concentrations are set at the 99th percentile of a healthy population and to separate between two disease states. Troponin is no longer an AMI marker but a marker of "myocardial injury". Minor increases in troponin concentrations are seen in a variety of non-AMI conditions that are associated with heart damage, such as heart failure, renal failure, sepsis, pulmonary embolism, and many others. CK-MB is a more specific test for AMI because levels are below the AMI cutoff concentration. This change in the strategy for cutoff concentrations has caused considerable confusion among emergency department physicians and cardiologists. Other factors besides cardiac biomarkers must be taken into consideration such as electrocardiographic tests, the clinical presentation of chest pain, and the patient's prior medical history. The demonstration of a rising troponin level over serial blood collections is also an essential part of the diagnostic workup.

The retention of CK-MB by clinical laboratories as a laboratory test for AMI may be due to the lack of knowledge and education as to the superior information that troponin produces. If this biomarker is only to be used as an AMI marker, a higher cutoff concentration can be selected, e.g., with ROC curve analysis, that differentiates between AMI and these other disease entities. In doing so, it would negate the advantages of troponin for early disease diagnosis and AMI rule out, and for risk stratification (prediction of future adverse cardiac events).

The need for early AMI diagnosis and rule out

In order to improve work flow and to reduce costs, there is a need by emergency department physicians to quickly triage patients to the appropriate level of care. Given the importance of cardiac biomarkers, there is significant interest in cardiac biomarkers that can detect early myocardial injury. CK-MB and myoglobin are cytoplasmic proteins and are released into the circulation from this pool following cardiac injury. Their appearance into blood is dictated by the size of the protein. Myoglobin at 17 kDa appears in blood within the first few hours after injury and returns to normal with 24 h. CK-MB at 84 kDa appears in blood a few hours after myoglobin and returns to baseline within 2–3 days.

There are two intracellular myocardial compartments for troponin. The free cytosolic pool accounts for 2–4% for cTnI and 6–8% for cTnT.[6] Following myocardial injury, these free subunits are initially released into the systemic circulation. (The secondary rise of troponin due to the breakdown of the structural elements are described in the next section.) Free troponin I and T have a molecular weight of 22 and 39 kDa, respectively. These are considerably smaller than CK-MB and are near that of myoglobin (at least for cTnI). Given the similarity in size, it would be expected that free troponin would appear in blood at or near the same time as myoglobin. From the previous literature, there is evidence and an ongoing perception that myoglobin and even CK-MB are earlier markers of cardiac injury than troponin (T or I). Tucker et al. examined all four markers on a group of patients presenting to the emergency department with chest pain and reported clinical sensitivities of 33%, 26%, 4% and 15%, respectively, for myoglobin, CK-MB, cTnI, and cTnT on blood collected at presentation.[7] Similar trends of better early diagnostic performance for myoglobin and CK-MB than troponin were reported by Mair et al.[8] (46%, 59%, 23%, and 28%, respectively). The poor showing of troponin was due to the insensitivity of first generation troponin assays. This was further suggested by studies conducted in healthy subjects, where earlier insensitive assays are unable to detect troponin in healthy subjects.[9] In both of these reports, very high cTn concentrations were used compared to the 99th percentile cutoffs used today (e.g., up to 600 ng/L for cTnI and 200 ng/L for cTnT). With the development of high sensitivity (hs) troponin assays and the use of the 99th percentile, the early detection of troponin is no longer limited by analytic insensitivity, and cutoff concentrations have been lowered to between 10 and 30 ng/L. Use of these highly sensitive troponin assays have enabled early rule out of AMI of 60% of patients as soon as 1 h after ED presentation.[10] This is only possible with the use of a hs-cTn assay, which enables the application of a combination of a very low absolute hs-cTn cutoff concentration (below the 99th percentile limit) coupled with a change in results from presentation to 1 h. Neither CK-MB nor myoglobin is used in the early assessment of these patients with use of hs-cTn assays.

There are also theories that troponin is released following reversible injury. Under this hypothesis, oxygen deficits caused by the occlusion of a coronary artery may release biomarkers into the circulation before the myocyte is irrevocably damaged. These theories for troponin are predicated by the observations that the early appearance of troponin in blood is not followed by the continued release from damaged myofibrils.[11] If this proves to be true, then troponin can be used to detect the onset of myocardial irreversible injury before it occurs. By pathophysiologic definition, ischemic markers will necessarily be earlier than markers thought to be released with myocardial necrosis. This too would obviate the need for use of CK-MB and myoglobin.

Detection of reinfarction

Some laboratorians may have retained CK-MB testing to identify an occasional case of re-myocardial infarction, a second usually more severe MI that occurs after the initial insult. In one study of 338 cases from Spain, the incidence of reinfarction was 3% of all AMIs and was associated with a higher mortality (37.8% versus 12.6%).[12] If a reinfarction occurs within 2–7 days after the initial infarct, there can be some diagnostic confusion with the use of cardiac troponin, and is worthy of explanation/discussion.

The largest fraction of troponin bound to the thin filament of muscle and part of a complex of three proteins (T, C, and I) (Fig. 1). Release of these proteins after injury requires breakdown of the myofibrillar unit. Therefore there is a delay in the appearance of these structural proteins into blood.[13] This biphasic release pattern can be confusing to cardiologists who wish to detect the presence of a "reinfarction." When this occurs, there is continued release of troponin from the initial infarction (patterns A vs. B of Fig. 1). Apple et al. compared the release of CK-MB with cTnI in 9