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Review

Analytical and assay issues for use of cardiac troponin testing for risk stratification in primary care

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ABSTRACT

Cardiac troponin is the standard marker for diagnosis of acute myocardial infarction and risk stratification of patients who present to an emergency department with signs and symptoms of acute cardiac ischemia. Over the past few years, the analytical sensitivity of assays for cardiac troponin has improved significantly to the point where a detectable amount of troponin can be measured in essentially all healthy subjects. Recent studies have shown that use of a highly sensitive troponin assays may provide value to traditional markers of primary disease risk for patients, i.e., for those who have no history of heart disease. There are barriers to the adoption of cardiac troponin for screening high risk cohorts such as the elderly, diabetics and perhaps even the asymptomatic population. Strategies used for the assignment of cutoff concentrations in acute care, i.e., the 99th percentile, may not be appropriate for primary care as changes over baseline levels may provide more accurate information of risk than cross-sectional results. A review of biological variation has shown that cardiac troponin as a biomarker has low index of individuality, indicating that reference values are of little utility. Whether or not cardiac troponin can be released in reversible injury is a debate that could have significance for detecting minor myocardial injury. A major hurdle for use of troponin in primary care is the lack of assay standardization and nomenclature for the different generations of troponin assays. Standardization requires knowledge of what is released after cardiac injury and what the various cardiac troponin assays are measuring. Currently it is not clear if the cardiac troponin release after ischemic injury is identical to that in circulation of healthy individuals. This may affect the design of future assays and standardization approaches. There is potential that a marker of myocardial injury such as troponin can add to the value of existing indicators and biomarkers of cardiovascular disease risk. Additional analytical and clinical validations are needed to fully elucidate cardiac troponin metabolism and resolve ongoing clinical and laboratory issues. While these issues are directed to the use of troponin in primary care, most of these concepts are relevant to the use of troponin in acute coronary syndromes as well.

Contents

Abbreviations: AMI, acute myocardial infarction; WHO, World Health Organization; CK, creatine kinase; cTnI and cTnT, cardiac troponin I and T; NACB, National Academy of Clinical Biochemistry; ROC, receiver operating characteristic; ESC, European Society of Cardiology; ACC, American College of Cardiology; AHA, American Heart Association; CV_A, CV_I, CV_G, coefficient of variance analytical, intraindividual, and interindividual; II, index of individuality; RCV, reference change value; IFCC, International Federation of Clinical Chemistry; SMCD, Standardization of Markers of Cardiac Damage; SI, International System of Units; SRM, standard reference material; RM, reference material; ISO, International Organization for Standardization.

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Introduction

The diagnosis of acute myocardial infarction (AMI) was established by the World Health Organization (WHO) and was predicated on finding two of three criteria: 1) clinical history of severe and prolonged chest pain, 2) unequivocal changes in the electrocardiogram, and 3) unequivocal changes in serial enzymes [1]. According to this definition, AMI could be diagnosed even in the absence of elevations in the cardiac enzymes. Enzymes such as aspartate aminotransferase, creatine kinase (CK), the CK-MB isoenzyme, lactate dehydrogenase (LD), and LD isoenzymes were most frequently used in 1979 when this report was written, and circulating forms of cardiac troponins I (cTnI) and T (cTnT) had not yet been discovered. cTnI and cTnT were investigated as potential biomarkers of AMI in 1987 [2] and 1989 [3], respectively.

In the early 1990's, cTnT and cTnI were approved for use in diagnosis of AMI by the US Food and Drug Administration. It was nearly10 years before these tests were fully sanctioned for standard practice by the scientific and medical communities. In 1999, The National Academy of Clinical Biochemistry (NACB) established the first set of guidelines for the use of cardiac markers including troponin in coronary artery diseases [4]. The NACB Guidelines determined the normal range as values above the 97.5th percentile of a healthy population (1-tailed test). This recommendation was consistent with the establishment of other clinical laboratory tests. For diagnosis of AMI, the NACB maintained the common practice that the cutoff concentration for the diagnosis of AMI be determined using receiver operating characteristic (ROC) curve analysis. This statistical method enables the selection of an optimum troponin value that separated patients with AMI from those patients suspected of AMI (e.g., those who presented with chest pain) but on whom a diagnosis of AMI was ruled out. As shown in Fig. 1, this ROC-derived cutoff is considerably higher than the 99th percentile cutoff recommended today. Finally, members of the NACB Committee also urged the WHO to update the definition of AMI to incorporate the use of cardiac biomarkers that were themselves not *enzymes*.

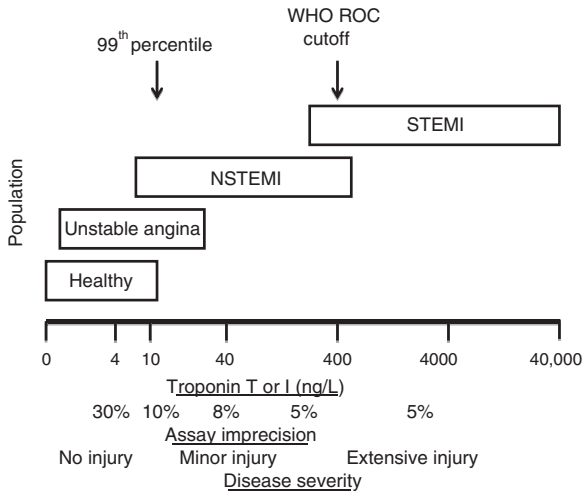

Fig. 1. Results of cardiac troponin in serum of various populations. The 99th percentile cutoff for the third generation cTnI assay is approximately 10 pg/mL and 13 ng/mL for the high-sensitivity troponin T assay.

On the heels of the NACB Guidelines, the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC) Committee redefined AMI in 2000 based on cardiac troponin [5]. The redefinition of an acute or evolving MI was predicated on a single cardiac troponin value exceeding the 99th percentile of a reference control population in the presence of either a) ischemic symptoms, b) development of pathologic Q waves on the electrocardiogram (ECG), c) ECG changes indicative of ischemia, and d) coronary artery intervention. Pathologic findings of an acute MI were an additional qualifying criterion. Importantly, this redefinition changed the AMI cutoff concentration from an ROC-derived value differentiating non-AMI from AMI cases to the 99th percentile of a healthy population, and increased from the 97.5th percentile recommended by the NACB. The ESC/ACC Committee also stated that the acceptable imprecision at this cutoff should be $\leq$10%. Unlike the WHO definition, where a diagnosis of AMI could be rendered with normal enzyme results but with definitive ECG changes, all patients with AMI must have an abnormal biomarker result, notably troponin.

In subsequent years, the Committee was expanded to include representatives from the American Heart Association and World Heart Federation, and in 2007 updated the redefinition of myocardial infarction [6]. For patients with a positive cardiac biomarker, the Task Force added criteria to the predicate biomarker (preferably troponin) including the presence of new left bundle branch block on the ECG, and positive imaging results (i.e., evidence of new loss of viable myocardium or regional wall motion abnormality). They also specified MI criteria following coronary artery intervention. For angioplasty and coronary artery bypass grafting, a troponin $> 3 \times$ 99th percentile and $> 5 \times$ 99th percentile, respectively, indicate a procedural-related MI. The Biochemistry Subcommittee of the Task Force recently opined that while a 10% imprecision for a cardiac troponin assay is ideal, the medical consequences of using less precise assays, e.g., between 10 and 20% at the 99th percentile is minimal, therefore these assays should not be precluded [7]. Apple et al. simulated the distribution of troponin results in a healthy population and introduced random assay imprecision to determine the number of serial results that falsely exceeded the 99th percentile [8]. The introduction of 25% imprecision resulted in additional 3 and 5 cases out of 1000 for the second and third measurements, respectively. While this imprecision added a relatively small number of false positives, the Subcommittee maintained that the use of troponin assays with imprecision greater than 20% was discouraged.

Release of troponin from damaged myocytes

The prevailing view by most cardiologists, pathologists, emergency department physicians, and clinical laboratorians is that cardiac troponin is only released following irreversible myocardial damage. Fig. 2A illustrates this concept, which is initiated by a lack of blood flow that causes anoxic injury. Disruption to the cell membrane follows which results in the release of low molecular weight free troponin subunits from the cytoplasm into the general circulation within the first hour(s) after onset. This is followed by the gradual breakdown of the myofibrils and release of the troponin complex that takes place over the ensuing 5–7 days [9]. Under this hypothesis, there is no release of troponin during the first few hours where injury to myocardial cells is reversible. The delay from the onset of chest pain to the appearance of troponin in blood was thought to be due to the time needed for the cells to overcome energy stores and anaerobic metabolism to become permanently