Research and Reviews: Journal of Journal of Cholesterol and Heart Disease

Extended Abstract

Vol. 2, Iss. 2

2018

Heart Congress 2017: High Sensitive Troponin as Biomarker for Coronary Artery Disease - M. Imteyaz Ahmad Department of Biochemistry, Sir Ganga Ram Hospital, New Delhi, India

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Introduction

Cardiac troponins are highly sensitive and specific biomarkers of myocardial necrosis and are used for the diagnosis of acute myocardial infarction. Troponin is a specific cardiac marker for stratification of diagnosis of coronary artery disease. Troponin assay combined with clinical symptoms of chest pain and other diagnostic finding helps in diagnosis of STEMI and NSTEMI.

Troponin consists of three subunits-troponin I, troponin T, and troponin C. Each subunit has a function: Troponin T binds the troponin components to tropomyosin, troponin I inhibits the interaction of myosin with actin, and troponin C contains the binding sites for Ca2+ that helps initiate contraction. Cardiac troponins are the products of different genes consisting of immunological distinct entities. The total quantity of troponin is very less compared to myofibrillar protein actin and myosin. Cardiac isoforms of troponin T and Troponin I are known to be cardiospecific and are increased in myocardial damage. Release kinetics following MI show a first peak due to loosely bound troponin pool and a second prolonged elevation due to degradation of the contractile apparatus [1]. cTnT have characteristic higher molecular weight, higher fraction of unbound cTnT, less degradation, whereas cTnI is more frequently found as binary or tertiary complex in blood. Previous studies proves that early appearing troponin pool may give information on the quality of microvascular reperfusion, while the concentration of cTn on Day 3 or 4 reflects myocardial infarct size [2].

The National Academy of Clinical Biochemistry issued a guideline in 2007 that stated that "in the presence of a clinical history suggestive of ACS, the following is considered indicative of myocardial necrosis consistent with myocardial infarction an abnormal value is that above the 99th percentile of the healthy population as a cut-off using an assay with acceptable precision.

The 99th percentile cutoَ و point for cardiac troponin T (cTnT) is well-known at 0.01 ng/mL (with 10% coeٹcient of variance value at the 99th percentile of 0.03 ng/mL), at least one occasion during the first 24 h after the clinical event" [3].

Different manufactures assay are commercially available for cardiac troponin I (cTnI), so the 99th percentile cut point varies based on the assay being used. It's recommended for each local laboratory to verify the reference cut off limits of the 99th percentile of a reference decision limit (medical decision cut off (for cardiac troponin (cTn) assays.

hs-cTn Assays

Rapid advancements in immunological assays have led to manufacturers to provide a traceable troponin calibration standards leading to improvement in analytical sensitivity and precision. Roche's Elecsys TnT Gen 5 STAT test was cleared by FDA recently is the first high sensitive troponin T assay. The fiih-generation blood test uses two monoclonal antibodies against cardiac troponin T to pick up the marker of myocardial damage with a turn-around time of 9 minutes are performed on Cobase 601 module of Roche fully automatic analyzer. The FDA clearance was based in part on a study of more than 1,000 patients with suspected acute MI in more than a dozen U.S. centers [4].

The hs-cTnT (fiih generation) assay cTnT assay uses fragment antigen-binding (FAB) of two c7n7-specific mouse monoclonal antibodies in a sandwich format. The capture antibody (M7) is biotinylated and directed against an epitope at amino acid residues 125–131 located in the central part of the cTnT molecule. The detection antibody is directed against an epitope at

This work is partly presented at World Heart Congress May 22-24, 2017 Osaka, Japan

amino acid residues 136–147. Detection of cTnT is based on an electrochemiluminescence immunoassay using a Tris (bipyridyl)- ruthenium (II) complex as a label. The original antibody (M11.7) has been genetically reengineered, with the constant C1 region of the FAB being replaced by a human IgG C1 region to produce a mouse–human chimeric detection antibody [5]. The rationale for this replacement was to further reduce the susceptibility to interference by heterophilic antibodies. The analytical sensitivity was improved by increasing the sample volume from 15 µL to 50 µL, increasing the ruthenium concentration of the detection antibody, and lowering the background signal via buffer optimization. The fifth generation assay is calibrated against recombinant human cTnT produced in Escherichia coli cell culture. Assay calibration is different from that of the fourthgeneration assay, so identical samples measured with the fourthgeneration and hs-cTnT assays will give different results. As a result of these modifications in label and antibody, the analytic performance of the hs-cTnT assay was significantly improved; specifically, the LoD was 0.003 ng/mL (3 ng/L), the 99th percentile cut-off point was 0.014 ng/mL (14 ng/L), and the CV was 10% at 0.013 ng/mL (13 ng/L). Due to a lower LoD and a increased precision, the hs-cTnT assay is able to detect more subtle elevations indicative of cardiac injury [6].

With the advent of hs-assays and the emphasis on imprecision (% CV) of assays at the 99th percentile URL and the limit of quantification (LoQ), along with the increasing role of using the limit of blank (LoB) and the limit of detection (LoD) as cut offs for early rule-out of AMI, understanding what these values mean is important all are analytical parameters used to describe the low concentrations of cardiac troponin measurements.

Total Imprecision (% CV) at 99th Percentile Day-to-day imprecision of cardiac troponin assays is defined by the % CV and is determined using multiple lots of both reagents and calibrators over multiple days [7]. The CLSI EP5-A2 document details the evaluation protocol that spans 20 days, 2 repeats a day, with 2 different lots used for reagents and calibration materials. For clinical use, cardiac troponin assays have been deemed “guideline acceptable” if they have a % CV of ≤ 10% at the 99th percentile, “clinically usable” if the % CV is >10% to ≤ 20%, and “not clinically acceptable” if the% CV is ≥ 20% [6,7]. The hs-assays meet the highest standard of clinical practice guideline precision recommendations (% CV < 10%) at the 99th percentile, whereas contemporary and POC cardiac troponin assays have a % CV between 10% and 20% at the 99th percentile. Using hscTn assays decreases analytical noise, allowing reporting of real cardiac troponin increases above the 99th percentile indicative of myocardial injury, rather than increases in cardiac troponin resulting from analytical imprecision, thereby improving diagnostic accuracy.

Statistical Approach to diffine 99th Percentile

Uniform, standardized statistical approach to calculate cardiac troponin 99th percentiles are required. The nonparametric method is a distribution-free method using ranks of observed values to determine percentiles for a given reference interval/cut off .It is simple to calculate and easy to determine a sample size. For a 99th percentile and 90% CI, n ≥ 299 study participants are required, as recommended by the IFCC TF-CB.

Implementing Δ Cardiac Troponin Values

There is no universal Δ value for cTnI or cTnT values to best optimize clinical specificity [8]. Deltas will be assay dependent and vary between a percentage change for contemporary and POC assays as compared to absolute concentration changes for hs-assays. Further, the calculation of Δ values will vary depending on the timing between serial samples, i.e., 0–1, 2 or 3 h (hs assays) vs 0 to 6 h (contemporary assays), as well as whether the initial cardiac troponin concentration is within the reference range below the 99th percentile (at the time of the disease evolution) or above the 99th percentile. Small concentration changes may also result from poor analytical imprecision for contemporary and POC assays that tend to have poor imprecision at low cardiac troponin concentrations, particularly those near the 99th percentile. A recommendation has been made for hs-cTnT to use a 50% change near the 99th percentile URL and a 20% change when the baseline value is increased above the

This work is partly presented at 27th International Conference on Clinical & Experimental Cardiology Research September 21-22, 2018 Vancouver, British Columbia, Canada.

99th percentile within a 3 h interval [9]. Shorter time intervals (≤ 3 h) will be required with hs-cTn assays to assist in ruling out AMI.

False Positive Cardiac Troponin Results

Analytical issues Falsely elevated troponin concentration due to analytical interference caused by various interferents. Fibrin clots in serum as a result of incompletely clotted specimen, e.g. in patients with coagulopathy or on anticoagulant therapy so Plasma is commonly used as specimen for analysis, heterophile antibodies, human anti-animal antibodies decreased due to use of higher generation immunoassay with improved specificity, rheumatoid factor, and autoantibodies, interference from other endogenous components in the blood such as bilirubin and hemoglobin interfere with absorbance, immunocomplex formation, microparticles in specimen, high concentration of alkaline phosphatase, analyzer malfunction are some of the commonly known (Table 1) [10-12].

Implementation of hs-Assays

The following draft check-list, derived from the evidence based literature, will help educate laboratories currently using and prepare laboratories not using hs-assays for the conversion from contemporary and POC assays to hs-assays. First, education of laboratory medicine and clinical staff on relevant literature pertaining to the manufacturers' assay being implemented will be necessary. Distribute analytical, diagnostic and risk assessment outcomes studies to your clinical colleagues, and present information on the new hs-assay at their staff meetings and clinical conferences. Second, develop understanding among users of the new hs-assay that the concentration numbers are going to change, and that they should not expect a conversion factor. Third, a URL at the 99th percentile will need to be established, following the IFCC TK-LB educational materials. Fourth, preparation will be needed for changing from a single to sex-specific 99th percentile, recognizing that this value for women will be less than for men.) fifth, conversion to reporting only whole numbers in ng/L will be needed. Sixth, define a QC material at the 99th percentile to monitor % CV. Seventh, consider using cardiac troponin values LoD of the hsassay as a potential rule-out characteristic. Eighth, provide serial testing protocols that consider earlier measurements such as at baseline, 1.5 h and 3 h for diagnostic determinations. Nineth work on assuring good preanalytical sampling as the hs-assays are so sensitive that poor sample quality can be a problem [14,15].

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