NACB Guidelines: Prepared by F Apple (032604) - Version 6

Chapter 3a: Analytical: ACS and Biomarkers - Cardiac Troponin and CKMB

I. **Overview of Topic**

In 2001, the IFCC C-SMCD established recommended quality specifications for cardiac troponin assays (1). The objectives were intended for use by the manufacturers of commercial assays and by clinical laboratories utilizing troponin assays. The overall goal was to attempt to establish uniform criteria in order that all cardiac troponin assays could objectively be evaluated for their analytical qualities and clinical performance These general principals can also be applied to CKMB mass and myoglobin assays. Both analytical and pre-analytical factors were addressed. This includes the following analytical recommendations. First, the antibody specificity as to what epitope locations are identified needs to be delineated. Epitopes located on the stable part of the cTnI molecule should be a priority. Specific relative responses need to be described for the following cTnI forms: free cTnI, the I-C binary complex, the T-I-C ternary complex, and oxidized, reduced and phosphorylated isoforms of the three cTnI forms. The effects of different anticoagulants on binding of cTnI need to be addressed. Second, the source of material used to calibrate cTn assays, specifically for cTnI, should be reported. Currently, a cTnI standardization subcommittee of the AACC is addressing the use of several materials to develop a primary reference material that will assist in at least harmonizing cTnI concentrations across different assays (2). The only way complete standardization will be possible for cTnI would be for all assay manufacturers to use

standard antibody pairs and a standard referefence material for calibration,. For cTnT however, as there is only one assay manufacturer (Roche Diagnostics), standardizing between assay generations has been consistent. Third, assays need to describe methods used for obtaining minimal detection limits (mean plus 3 SD of 20 replicates of a zero calibrator) and total imprecision; describing at what concentration a 10% CV is attained. Pre-analytical factors that should be described include effect of storage time and temperature, effect of glass vs. plastic tubes and gel separator tubes, and influence of anticoagulants and whole blood measurements. As more assay systems are devised for POC testing, the same rigors applied to the central laboratory methodologies need to be adhered to by the POC testing systems.

While clinicians and laboratorians continue to publish guidelines supporting TATs of < 60 minutes for cardiac biomarkers, the largest TAT study published to date has demonstrated that TAT expectations are not being met in a large proportion of hospitals. A CAP Q probe survey study of 7020 cardiac troponin and 4368 CKMB determinations in 159 hospitals demonstrated that the median and 90th percentile TAT for troponin and CKMB were as follows: 74.5 min, 129 min; 82 min, 131 min; respectively (3). Less than 25% of hospitals were able to meet the < 60 minutes TAT, representing the biomarker order-to-report time. Unfortunately, a separate subanalysis of just POC testing systems was not reported. Preliminary data has shown that implementation of POC cardiac troponin testing can decrease TATs to < 30 minutes in cardiology critical care and short stay units (4). These data highlight the continued need for laboratory services and health

care providers to work together to develop better processes to meet a < 60 min TAT as requested by physicians.

Several markers should no longer be used to evaluate cardiac disease. These include aspartate aminotransaminase (AST), total lactate dehydrogenase (LD), and LD isoenzymes. These markers have poor specificity for the detection of cardiac injury because of their wide tissue distribution. Because total CK and CKMB have served as standards for so many years, some laboratories may continue to measure it to allow for comparisons to cardiac troponin over time, before discontinuing use of CK. In addition, the use of total CK in developing countries may be the preferred and only alternative for financial reasons. CK can also assist in improving myocardial tissue specificity when the ratio of CKMB to total CK is > 2%. This concept is emphasized in a statement from the AHA Council on Epidemiology and Prevention, regarding case definitions for acute coronary heart disease in epidemiology and clinical research studies (5). To more accurately interpret recent trends in heart disease, specifically AMI, during the spread of new technology and the new definition of AMI predicated on cardiac troponin, the following recommendations were made: 1) simultaneous use of old biomarkers with cardiac troponin to determine the effects of new biomarkers and 2) consider adjustment factors in databases and retrospective studies seeking to determine incidence and trends of AMI before and after cardiac troponin-derived research studies.

Defining the 99th percentile of a reference group for cardiac troponin assays should be determined in each local laboratory by internal studies using the specific assay used in

clinical practice or validate the assay based on findings in the literature (5,6,7). Acceptable imprecision (coefficient of variation, %CV) of each cardiac troponin assay (as well as for CKMB mass assay) has been defined as $\leq$10% CV at the 99th percentile reference limit (5,6,7). Unfortunately, the majority of laboratories do not have the resources to perform adequately powered reference range studies nor the ability to carry out NCCLS protocols to establish total imprecision criteria for every cardiac troponin assay in the market place. Therefore, clinical laboratories need to rely upon the peer-reviewed published literature to assist in establishing local reference limits.

Caution must be taken when comparing the findings reported in the manufacturer's FDA cleared package inserts, with the findings reported in journals because of differences in total sample size, distributions by gender and ethnicity, age ranges, and the statistic used to calculate the 99th percentile given. To date, very few in-vitro diagnostic companies have published their 99th percentile cutoffs in their package inserts. There is no established guideline set by the FDA to mandate a consistent evaluation of the 99th percentile reference limit for cardiac troponins. The largest and most diverse reported reference range study to date shows plasma (heparin) 99th percentile reference limits for 8 cardiac troponin assay (7 cTnI, 1 cTnT) and 7 CKMB mass assays (8). These studies were performed in 696 healthy adults (age range 18 to 84y) stratified by gender and ethnicity. There was a 13-fold difference between the lowest vs. the highest measured cTnI 99th percentile limit. The lack of cardiac troponin assay standardization (there is no primary reference material available) and the differences in antibody epitope recognition between assays (different assays use different antibodies) give rise to substantially

discrepant concentrations. What is generally recognized, though, it that as long as one understands the characteristics of an individual assay, and does not attempt to compare absolute concentrations between different assays, clinical interpretation should be acceptable for all assays.

For CKMB, as has been recognized for years for total CK, all assays demonstrate a significant 1.2 to 2.6-fold higher 99th percentile for males vs. females (8). Further, there were several assays that showed higher, up to 2.7-fold, concentrations for African Americans vs. Caucasians. These data demonstrate that clinical laboratories must consider establishing different CKMB reference cutoffs for population subgroups, at least for men vs. women.

For cardiac troponin, how to implement reference cutoffs, at present, is not so clear. Since only one or two of the more than dozen commercially available assays can attain a total 10% CV at the 99th percentile, caution must be considered in implementing a diagnostic cutoff that may potentially give false positive analytical results. While the literature has been enriched with studies appropriately addressing the total imprecision of cTn assays, as to what the lowest concentration will be to attain a 10% CV, the manufacturers' package inserts prefer to publish imprecision data primarily based on within run or within day precision. Again, there is no consistent FDA specification regarding what precision value that should be reported in the package insert.

To better address day-to-day clinical laboratory practice, the IFCC C-SMCD has published findings demonstrating the total imprecision for 13 commercial assays, based on a 20-day NCCLS protocol (9); showing that no assay was able to experimentally achieve a 10% CV at their 99th percentile cutoff. Therefore, to avoid the potential for a false positive diagnostic criteria based on cTn monitoring at the 99th percentile, experts in both the laboratory medicine and cardiology communities have endorsed the concept that, until cardiac troponin assay imprecision improves at the low end, the lowest concentrations to attain a 10% CV should be used as a modified ESC/ACC diagnostic cutoff for detection of myocardial injury (6,7). The ultimate goal will be to have all cTn assays attain a 10% CV at the 99th percentile reference limit. This approach should reduce false positive analytic results from lack of imprecision values between the 10% CV cutoff and the 99th percentile. However, all biomarker increases above the 99th percentile should be interpreted cautiously, within the clinical context of the patient, and followed with serial samples over a 6 to 12h period after presentation.

For clinical trials, to avoid the confusion of multiple centers using multiple assays, several approaches are recommended for utilizing cTn testing (6,7). First, analyze all samples from trial centers in a core, central lab with a precise, well-defined assay. Second, provide all trial centers with the same well-defined assays. Third uniformly define each center's assays by using the 10% CV concentration (assay-dependent); thus, not relying on local lab criteria and troponin cutoffs. Fourth, use a multiple (2 to 3-fold) of the 10% CV cutoff value. Fifth, if trials decide to use cutoff values defined in earlier studies, the degree of variability should be reported.

An ESC/ACC consensus conference along with the AHA/ACC guidelines for differentiating AMI and unstable angina codified the role of cardiac troponin by advocating that the diagnosis of AMI and establishing a high-risk profile (evidence of myocardial injury) is based on increases of cardiac troponin I or T (preferred) or CKMB, in the appropriate clinical situation (4,10,11). The guidelines recognized the reality that neither the clinical presentation nor the ECG had adequate clinical sensitivity and specificity. The guidelines do not suggest that all increases of these biomarkers should elicit a diagnosis of AMI or high-risk profile; only those associated with the appropriate clinical and ECG findings. When cardiac troponin increases not due to acute ischemia occur, the clinician is obligated to search for another etiology for the elevation.

II. **Recommendations**

1. Cardiac troponin (I or T) is the reference biomarker for the detection of myocardial injury, risk stratification in ACS, and for the diagnosis of acute MI. CKMB testing (mass assay preferred) is an acceptable biomarker for laboratories that are unable to perform cardiac troponin testing. There is no longer a role for monitoring AST or lactate dehydrogenase (total activity or isoenzymes) for the detection of myocardial injury of AMI.

Strength/consensus of recommendation: Class I a

2. Reference decision limits should be established for each cardiac biomarker based on a population of normal, healthy individuals, without a known history of heart disease. For cTnI, cTnT, CKMB mass and myoglobin the 99th percentile should be the decision limit for myocardial injury. Gender specific reference limits should be used in clinical practice for CKMB mass.

Strength/Consensus of recommendation: Class I a

3. One decision limit is recommended for the optimum use of the cardiac biomarkers cTnI, cTnT and CKMB mass. ACS patients with cTnI, cTnT or CKMB mass results increased above the reference decision limit should be labeled as having myocardial injury and a high risk profile. These patients should be admitted and considered for treatments and interventions for reducing short ($\leq$ 30days)- and long ($\geq$ 6 months)-term risks of adverse events (death, MI or both).

Strengths/Consensus of recommendation: Class I a

4. In the clinical setting of ischemia, increases in cTnI, cTnT, or CKMB above the decision limit qualifies as an acute myocardial infarction (AMI) as defined by the ESC, ACC and AHA.

Strengths/Consensus of recommendation: Class I a

5. When neither cardiac troponin I or T or CKMB are available, monitoring total CK or CKMB activity for the diagnosis of acute MI or myocardial injury is acceptable.

However, the lack of (myocardial tissue) specificity for these biomarkers needs to be acknowledged.

Strength/Consensus or recommendation: Class II c

6. Assays for cardiac biomarkers should have a total imprecision (%CV) of $\leq$10% at the 99th percentile reference limit. Before introduction into clinical practice, biomarker assays must be characterized with respect to potential interferents, including rheumatoid factors, human anti-mouse antibodies, heterophile antibodies, and other related proteins. Preanalytical and analytical assay characteristics established should include biomarker stability (over time and across temperature ranges) for each acceptable specimen type used in clinical practice, and identification of antibody/epitope/recognition sites for each biomarker. IFCC specifications should be followed.

Strength/Consensus of recommendation: Class II c

7. Serum, plasma or anticoagulated whole blood are accetable specimens for the (ASAP) analysis of cardiac biomarkers. The choice of specimen will be dependent upon the known characteristics of individual biomarker assays regarding how each specimen type performs.

Strength/Consensus of recommendation: Class II c

8. Receiver operator characteristic (ROC) curves should be used to evaluate the clinical effectiveness of each cardiac biomarker, using a cardiac troponin assay as the predicate biomarker..

Strengths/Consensus of recommendation: Class II b

9. Early in the research and development process, manufacturers should seek guidance from and provide support to professional organizations within Laboratory Medicine, Cardiology (AHA, ACC, ESC) and Emergency Medicine (SAEM) to establish committees to initiate standardization of new cardiac biomarkers and establish acceptable, clinically relevant, preanalytical characteristics for biomarker assays.
Strength/Consensus of recommendation: Class I c

10. The laboratory should perform, ASAP, cardiac biomarker testing on a continuous 24 hour basis, with a TAT of < 60 minutes; optimally at 30 minutes. The TAT is defined as the time from blood collection to clinician or caregiver awareness.
Strength/Consensus of recommendation: Class II b

11. Institutions that cannot deliver cardiac biomarker TAT in < 60 minutes should consider implementation of POC testing systems. Performance characteristics, as defined by the IFCC quality specification criteria for cardiac troponin, should not be different between central laboratory and point of care (POC) biomarker testing platforms. An acceptable harmonization of POC and central laboratory results should be $\leq$20%.
Strength/Consensus of recommendation: Class II c

12. Pertaining to implementation of POC testing of cardiac biomarkers, laboratory personnel must be involved in:

Selection of devices

Training of individuals performing the analysis

Maintenance of POC equipment

Verification of the proficiency and competency of operators on a regular basis

Compliance of documentation with requirements by appropriate agencies

Connectivity issues regarding interfacing POC equipment to the Laboratory Information System (LIS)

In meeting these requirements, a quality assurance and quality control program must be instituted and fully documented on a regular basis. A procedure must be in place to address actions that will be instituted when non-compliance with appropriate POC testing processes occur.

Strength/Consensus of recommendation: Class II c

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