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National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: Analytical Issues for Biochemical Markers of Acute Coronary Syndromes

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I. Overview of Analytical Issues for Acute Coronary Syndrome (ACS) Biomarkers

A. Background

In 1999, the National Academy of Clinical Biochemistry (NACB)[6] published the first standards of laboratory practice addressing analytical and clinical recommendations for use of cardiac markers in coronary artery diseases *(1)*. The objectives were to recommend the appropriate implementation and utilization of cardiac biomarkers, specifically for cardiac troponin (cTn), which had just gained US Food and Drug Administration (FDA) clearance as a cardiac biomarker to aid in the diagnosis of acute myocardial infarction (AMI). In 2001, the IFCC Committee on Standardization of Markers of Cardiac Damage (C-SMCD) recommended quality specifications for analytical and preanalytical factors for cTn assays *(2)*. The objectives were intended for use by the manufacturers of commercial assays and by clinical laboratories that use cTn assays. The overall goal was to establish uniform criteria so that all cTn assays could objectively be evaluated for their analytical qualities and clinical performance. These general principles can also be applied to creatine kinase MB (CK-MB) mass and myoglobin assays by use of the analytical recommendations in this document. In this report, we provide the background for establishing updated practice guidelines with recommendations addressing analytical issues for cardiac biomarkers based on 8 years of evidence-based medical and scientific observations since the publication of the initial recommendations *(1)*.

II. Analytical Biomarker Issues

RECOMMENDATIONS: ANALYTICAL ASPECTS OF ACS BIOMARKERS

ALL CLASS I

1. 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 (reference population). For cardiac troponin I (cTnI) and T (cTnT), as well as for CK-MB mass, the 99th percentile of the reference population should be the decision-limit for myocardial injury. The Clinical Laboratory Standards Institute (CLSI; formerly NCCLS) recommends a minimum of 120 individuals per group of healthy individuals for appropriate statistical determination of a normal reference limit cutoff.

   Sex-specific reference limits should be used in clinical practice for CK-MB mass. For myoglobin, the 97.5th percentile (with sex-specific reference limits) should be the decision-limit for myocardial injury (Level of Evidence: B).
2. One decision-limit, the 99th percentile, is recommended as the optimum cutoff for cTnI, cTnT, and CK-MB mass. ACS patients with cTnI and cTnT results above the decision-limit should be labeled as having myocardial injury and a high-risk profile (Level of Evidence: B).
3. Assays for cardiac biomarkers should strive for a total imprecision (%CV) of ≤10% at the 99th percentile reference limit. Before introduction into clinical practice, cardiac biomarker assays must be characterized with respect to potential interferences, including rheumatoid factors, human anti-mouse antibodies, and heterophile antibodies. Preanalytical and analytical assay characteristics 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. Analytical and preanalytical specifications developed by professional groups such as the IFCC should be followed (Level of Evidence: C).
4. Serum, plasma, and anticoagulated whole blood are acceptable specimens for the analysis of cardiac biomarkers. Choice of specimen must be based on sufficient evidence and the known characteristics of individual biomarker assays (Level of Evidence: C).

A. cTn specifications

First, in the context of cTn, the epitopes recognized by the antibodies must 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 3 cTnI forms. The effects of different anticoagulants on binding of cTnI also need to be addressed. Second, the source of material used to calibrate cTn assays, specifically for cTnI, should be reported. A cTnI standardization subcommittee of the AACC in collaboration with the NIST has developed a primary reference material (SRM #2921) *(3)*. Although this material demonstrated commutability with only 50% of current cTn assays, it will be of use in harmonizing cTnI concentrations across different assays *(4, 5)*. At present, it appears that the only way to achieve complete

[6] Nonstandard abbreviations: NACB, National Academy of Clinical Biochemistry; ACS, acute coronary syndrome; cTn, cardiac troponin; FDA, US Food and Drug Administration; AMI, acute myocardial infarction; C-SMCD, Committee on Standardization of Markers of Cardiac Damage; CK-MB, creatine kinase MB; cTnI, cardiac troponin I; cTnT, cardiac troponin T; CLSI, Clinical Laboratory Standards Institute; POC, point-of-care; TAT, turnaround time; MI, myocardial infarction; AHA, American Heart Association; ESC, European Society of Cardiology; ACC, American College of Cardiology; and WHF, World Heart Federation.

standardization for cTnI would be for all manufacturers to agree on using the same antibody pairs for all commercial assays as well as a common reference material for calibration *(6, 7)*. The IFCC C-SMCD is currently exploring the development of a serum-based secondary reference material. For cTnT, as there is only one assay manufacturer, harmonizing between assay generations has been consistent. Third, manufacturers need to use methods advocated by the CLSI to characterize detection limit, functional sensitivity, and total imprecision *(8, 9)*. Key characteristics for cTn assays include determination of the distribution of values in a healthy reference population, the statistical determination of the 99th percentile cutoff for the reference population, and determination of the concentration corresponding to the 10% CV (total imprecision). Preanalytical factors that should be described include effect of storage time and temperature, effect of glass vs plastic tubes and gel separator tubes, and the influence of anticoagulants for plasma and whole-blood measurements. As more assay systems are devised for point-of-care (POC) testing, identical analytical criteria must apply to both central laboratory methodologies and POC testing systems. When measuring cTn by different methodologies within the same institution, assay results should be harmonized or a strategy implemented to avoid interpretative confusion by clinicians.

B. CARDIAC BIOMARKER TURNAROUND

Clinicians and laboratorians continue to support a goal for turnaround times (TATs) <60 min for cardiac biomarkers, but the largest study published to date has demonstrated that TAT expectations are not being met in a large proportion of hospitals *(10)*. A College of American Pathologists Q-probe survey study of 7020 cTn and 4368 CK-MB determinations in 159 predominantly North American hospitals demonstrated that the median and 90th percentile TATs for troponin were 74.5 and 129 min, and for CK-MB, 82 and 131 min. In fact, fewer than 25% of hospitals were able to meet the <60-min TAT, defined as order-to-report time. A separate subanalysis of just POC testing systems was not reported. Recently published data have shown that implementation of POC cTn testing can decrease TATs to <30 min in cardiology critical-care and short-stay units *(11)*. These data highlight the continued need for laboratory services and healthcare providers to work together to develop better processes to meet a <60-min TAT as requested by physicians.

C. BIOMARKERS NO LONGER RECOMMENDED FOR USE IN THE EVALUATION OF ACS

Use of aspartate aminotransaminase, total lactate dehydrogenase, and lactate dehydrogenase isoenzymes are not recommended for evaluation of cardiac injury and detection of myocardial infarction (MI). The use of total CK or CK-MB activity is an acceptable alternative for evaluating cardiac injury in institutions where cTn or CK-MB mass assays are not available or feasible. Total CK can also assist in improving myocardial tissue specificity when the ratio of CK-MB to total CK is greater than previously established reference intervals. This concept is emphasized in a statement from the American Heart Association (AHA) Council on Epidemiology and Prevention regarding case definitions for acute coronary heart disease in epidemiology and clinical research studies *(12)*. The following recommendations were made to allow for a more accurate interpretation of recent trends in ACS during implementation of cTn assays and use of the European Society of Cardiology (ESC)/American College of Cardiology (ACC) consensus MI definition *(13, 14)* predicated on cTn: (*a*) simultaneous use of traditional biomarkers with cTn to determine the performance of new biomarkers; and (*b*) use of adjustment factors in databases and retrospective studies seeking to determine incidence and trends of MI before and after cTn–derived studies.

D. DETERMINING BIOMARKER DECISION CUTOFF CHARACTERISTICS FOR ACS

The 99th percentile of a reference decision-limit (medical decision cutoff) for cTn assays should be determined in each local laboratory by internal studies using the specific assay that is used in clinical practice or validating a reference interval that is based on findings in the literature *(13, 16)*. Desirable imprecision (expressed as %CV) of each cTn assay (and CK-MB mass assay) has been defined as ≤10% CV at the 99th percentile reference limit *(13, 16)*. Unfortunately, the majority of laboratories have neither the resources to perform adequately powered 99th percentile reference studies nor the ability to carry out CLSI protocols to establish total imprecision criteria for the cTn assay that they plan to use in practice *(17)*. Therefore, clinical laboratories must rely on the peer-reviewed published literature to assist in establishing both local reference limits and imprecision characteristics. Caution must be taken when comparing the findings reported in the manufacturers' package inserts, which have been cleared by the FDA, with the findings reported in journals because of differences in total sample size, distributions by sex and ethnicity, age ranges, and statistical methods used to calculate the 99th percentile reported.

To date, very few in vitro diagnostic companies have published 99th percentile limits in their package inserts. There is no established guideline set by the FDA or other regulatory agencies to mandate a consistent evaluation of the 99th percentile reference limit for cTns. The largest and most diverse reported reference value study to date shows plasma (heparin) 99th percentile reference limits for 8 cTn assays (7 cTnI and 1 cTnT) and 7 CK-MB mass assays *(18)*. This study was performed in 696 healthy adults (ages 18–84 years) stratified by sex and ethnicity. There was a 13-fold difference between the lowest vs the highest measured cTnI 99th percentile limit. The lack of cTn assay standardization (there is no primary reference material that is commutable with all commercial methods, as noted earlier) and the differences in antibody epitope

recognition between assays (different assays use different antibodies, as noted earlier) give rise to substantially discrepant concentrations. What is generally recognized, though, is 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 CK-MB (as has been recognized for years for total CK), all assays demonstrate a significant 2- to 3-fold higher 99th percentile limit for men vs women *(18)*. Further, CK-MB can demonstrate up to 2- to 3-fold higher concentrations for African Americans vs Caucasians—differences attributed to between-race physiological differences in muscle mass. These data led to the class I recommendation that clinical laboratories should establish different CK-MB reference limits based on sex. Labs should also consider doing so for ethnic groups.

For cTn, expert consensus has emerged in support of the 99th percentile as the reference cutoff, in spite of whether the total imprecision of the assay is ≤10%. This has been supported by a recent study that has demonstrated that misclassification of patients who are ruled out using cTn assays with variable imprecision (10%–25%) at the 99th percentile does not lead to significant patient misclassification over serial cTn orders *(19)*. Further, whereas 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 often publish imprecision data primarily based on within-run or between-day precision. Again, there is no consistent regulatory specification regarding precision data that should be reported in the manufacturers' package inserts. To better address day-to-day clinical laboratory practice, early findings from an IFCC C-SMCD study demonstrated that the total imprecision for 13 commercial assays [based on a 20-day CLSI protocol *(20)*] was unable to experimentally achieve a 10% CV at their 99th percentile limit. Improved 2nd-generation assays, however, have recently demonstrated 10% CVs at the 99th percentile *(21)*. The ultimate goal will be to have all cTn assays attain a 10% CV at the 99th percentile reference limit to reduce any potential of false-positive analytic results attributable to imprecision in the low concentration range.

For clinical trials, to avoid the confusion of multiple centers using multiple assays, several approaches are recommended for cTn testing *(15, 16)*. 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, FDA-cleared assay. Third, uniformly define each center's assays by using the 99th percentile concentration (assay-dependent), thus reducing reliance on local laboratory criteria for cTn decision cutoffs. Fourth, use a multiple (2- to 3-fold) of the 99th percentile. Fifth, if trials decide to use cutoff values defined in earlier studies, the degree of imprecision at these concentrations should be reported.

E. European Society of Cardiology/American College of Cardiology Recommendations

An ESC/ACC consensus document along with the AHA/ACC guidelines for differentiating AMI and unstable angina codified the role of cTn by advocating that the diagnosis of AMI be based on increases of cTnI or T (preferred) or CK-MB mass above the 99th percentile cutoff in the appropriate clinical situation *(14, 22)*. The guidelines recognized the reality that neither the clinical presentation nor the electrocardiogram had adequate clinical sensitivity and specificity. The guidelines do not suggest that all increases of these biomarkers should elicit a diagnosis of MI or high-risk profile, only those associated with the appropriate clinical, electrocardiogram, imaging, or pathological findings. When cTn increases are not due to acute ischemia, the clinician is obligated to search for another etiology for the elevation *(6, 23)*. Updated guidelines addressing the revised universal definition of MI cosponsored by the Joint ESC-ACC-AHA-World Heart Federation (WHF) Task Force For The Redefinition of MI will soon be published. This document will support and coincide with the recommendations proposed in the current joint NACB IFCC document.

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