Evaluation of High-Sensitivity Assays for Cardiac Troponin

Despite the facts that cardiac troponin was introduced 2 decades ago and has been established since 2000 as the preferred biomarker for the diagnosis of myocardial infarction (MI)\(^1\) (1), the clinical application of cardiac troponin assays continues to evolve substantively. In large part, this evolution has been driven by sustained progress in the analytical performance of commercially available cardiac troponin assays. Because of improved precision at low cardiac troponin concentrations and data establishing the prognostic relevance of quantitatively minor increases in this biomarker, the clinical decision limit for cardiac troponin has been pushed progressively lower. A recently emerged new generation of research assays for cardiac troponin now has reduced the limit of detection by another 10- to 100-fold compared with current commercially available assays (2, 3). In this issue of \textit{Clinical Chemistry}, the report by Eggers et al. (4) and 2 reports by Wu et al. (5, 6) provide valuable insights into possible new applications for more sensitive assays, as well as raise new questions that must be addressed in the course of evaluation of a new generation of high-sensitivity cardiac troponin assays.

\textbf{Where Are We Now?}

Because cardiac troponin is superior to the previous gold standard (creatine kinase isoenzyme MB) for MI diagnosis, the initial efforts to establish a uniform diagnostic decision limit were difficult and at times controversial. This challenge was (and still is) compounded by a lack of standardization of the multiple commercial assays for cardiac troponin I (cTnI) and the poor precision of some assays around the proposed diagnostic cutoffs; however, the uncertainty regarding the clinical significance of increases in cardiac troponin that are below the concentration equivalent to an abnormal increase in creatine kinase MB has been resolved by consistent epidemiologic data that have established a relationship between “low-level” increases in cardiac troponin among patients with suspected acute coronary syndrome (ACS) and the risk of subsequent major cardiovascular events (7). These observations have supported the current recommendations of professional societies for a single decision limit for MI diagnosis and risk stratification at the 99th percentile of a reference population for each assay (7, 8). Despite this clear recommendation and evidence establishing therapeutic implications at this cutoff, we are surprised to see in our practice that a substantial proportion of medical centers have maintained the approach of reporting more than one decision limit and/or one that is higher than the 99th percentile. For this reason, a necessary first step is to achieve a more widespread implementation of the present guidelines before embracing a consideration of cutoffs below the 99th percentile, as discussed later in this editorial with regard to the report of Eggers et al. (4).

\textbf{Where Are We Going?}

In a word, lower. First, improvements in the analytical performance of commercial assays have moved the limit of detection and enabled resolution of the 99th percentile at lower concentrations. Currently, with each iterative change toward lower cutoffs, clinical studies have confirmed a greater clinical sensitivity for MI diagnosis, earlier detection after symptom onset, and enhanced risk stratification when an assay is coupled with a clinical syndrome consistent with myocardial ischemia (7). For example, in a study of patients with definite myocardial injury but with an initially negative cTnI result as measured with a prior-generation commercial assay, analysis of samples from these patients with a newer assay with an improved functional sensitivity revealed detectable cardiac troponin with the first sample in 64% of the cases (9).

Second, an investigation of cutoffs below the 99th percentile has revealed an association between increases in cardiac troponin higher than the limit of detection and adverse cardiovascular outcomes (10). As an example, Eggers and colleagues studied a cohort of 952 patients stabilized after ACS and found that a cutoff set at the 90th percentile of the reference population for this study provided an improved capability to predict 5-year mortality, compared with the 99th percentile (4). This study also adds to prior work demonstrating that low cardiac troponin concentrations detectable with commercially available assays or enhanced commercial assays hold prognostic significance in settings other than initial presentation with ACS.

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\(^1\) Nonstandard abbreviations: MI, myocardial infarction; cTnI, cardiac troponin I; ACS, acute coronary syndrome.
tectable low concentrations of cTnI were associated both with prevalent coronary heart disease and with incident coronary events in patients free of symptomatic coronary heart disease (11). Moreover, in a stable general population at risk for coronary heart disease, Wallace and colleagues demonstrated that a detectable cTnI concentration was associated with a higher prevalence of risk factors and evidence of structural heart disease (12). Additionally, stable patients with chronic heart failure who have low plasma concentrations of cardiac troponin are at higher risk for death and for first hospitalization for heart failure (13). Together, these studies have established the concept that even minimally increased concentrations may represent subclinical cardiac injury and thus may have important clinical implications.

Third, emerging research assays for troponin have produced detection limits and reproducibility that are improved by at least one order of magnitude over present commercial assays (2,3). As an example, Wu et al. reported a CV of 10% at a troponin concentration of 0.0018 μg/L with their single-photon fluorescence-detection method (2). This new generation of assays offers the potential to characterize a cardiac troponin distribution in the healthy population, to detect small changes in troponin concentration over time that may assist in distinguishing acute myocardial injury from chronic causes of troponin release, and to expand the potential applications described in preliminary work with high-performing commercial assays. In addition, the reports of Eggers et al. and Wu et al. (4–6) in this issue highlight several important issues that merit consideration as the field approaches another potentially important step forward.

Challenges Ahead

First, with the rapid ongoing advances in cardiac troponin assays, defining an appropriate terminology to categorize past, present, and future assays in a manner that integrates all facets of performance and that will be useful to the laboratorian and clinician is not straightforward. For this editorial, we have taken license to describe the emerging generation of cardiac troponin assays as “high sensitivity”; however, precise definitions for this and other terms are under development.

Second, although there are now clear guidelines that establish the 99th percentile of “a normal reference population” as the diagnostic decision limit for MI (8), consistent guidelines for what constitutes a “normal reference population” have not been established. Eggers and colleagues (4) have confirmed that the selection of the reference population strongly influences the determination of the 99th percentile, even among apparently healthy individuals, a finding that previous work had forecast with a less sensitive assay (12). Moreover, some might argue that the optimal reference population could depend on the clinical application; for example, for diagnosis of MI among all visitors to an emergency department with chest pain vs for screening for subclinical structural heart disease in a stable outpatient population. High-sensitivity cardiac troponin assays will both magnify the need and provide a tool for refining our concept of “normal” via their use in testing a variety of stable populations, including those patients who have been exonerated of structural heart disease with the best available technology.

Third, as the limit of detection decreases with each generation of troponin assays, it will be necessary to reevaluate potential sources of variation that have not been meaningful with present commercial assays. It is possible that at the very low cardiac troponin concentrations detected with assays such as that of Wu et al., low-level nonspecific binding to other serum or plasma constituents will confound assay results. Indeed, Wu et al. (5) observed low-level nonspecific binding in up to 25% of samples with results below the lower limit of quantification, with 1 of 20 samples having a quantifiable result. The authors concluded that given the very low level of binding, the overall specificity of the assay tested was maintained. Nevertheless, this finding necessarily draws attention to the possibility of contributions from nonspecific binding at very low cardiac troponin concentrations. This influence, however, will be inherently accounted for during determination of the 99th percentile in reference populations. A second potential source of variation to be considered as the limit of detection moves orders of magnitude lower is the contribution of biological variation in cardiac troponin concentration over time. Wu and colleagues found within-day and between-day intraindividual CVs of 9.7% and 14.1%, respectively, for healthy individuals. This magnitude of variation was similar to the analytical CV and was <40% of the total CV for this population. Therefore, the intraindividual variation within a stable healthy population appears to be small relative to the differences between individuals. This important finding supports the concept that at very low concentrations, assessment of serial changes in the individual patient will be more useful than applying a general population-based reference cutoff. This concept is reinforced by the finding of Eggers et al. of an important influence of population characteristics on the determined 99th-percentile reference value.

Fourth, as higher-sensitivity cardiac troponin assays become incorporated into clinical practice, the number of patients who present with ACS and have diagnosed MI will increase. Similarly to issues faced during the transition from the use of creatine kinase MB to cardiac troponin (1), the implications of this
change will need to be considered with regard to the epidemiology of MI, including both its incidence and fatality rates, as well as societal implications for insurance and employment. Adequately powered clinical studies will also be needed to assess whether the therapeutic implications of low cardiac troponin concentrations measured with a high-sensitivity assay are the same as those established with current commercial assays. On the basis of the prior experience with each evaluation of lower decision limits to date (7), we expect that treatment with potent antithrombotic therapy and coronary intervention will benefit patients with a clinical syndrome consistent with ACS and an abnormal result in a high-sensitivity assay. Studies to confirm this hypothesis are warranted, however. Moreover, will hospitalization be necessary for those patients with a low clinical probability of ACS and a positive result in a high-sensitivity cardiac troponin assay, or will algorithms be developed that will allow for safe outpatient evaluation with close follow-up? Evidence-based answers to these questions will be important for formulating future clinical practice guidelines and performance measures that will be derived from them.

Lastly, although professional societies have recognized the importance of the enhanced analytical performance of the newer and emerging cardiac troponin assays (14), the clinical community has not uniformly embraced this trend. The application of assays with lower limits of detection also has led predictably to increases in the proportion of patients evaluated in the emergency setting who have detectable cardiac troponin concentrations in a variety of acute and chronic medical conditions other than ACS (9). From a prognostic perspective, it is noteworthy that in most of the settings studied to date, patients presenting with an increased cardiac troponin concentration have had poorer prognoses than those without detectable cardiac troponin. Nevertheless, this circumstance has at times led to frustration by the clinicians who must grapple with the test results. As illustrated by Eggers et al. and Wu et al., the emergence of high-sensitivity cardiac troponin assays is likely to open new doors for clinical application in a variety of settings in which patients may manifest myocardial injury. At the same time, such assays will demand a deeper understanding of the potential sources of “normal” and pathologic variation between and within patients that become relevant only at the very low cardiac troponin concentrations detected with the new assays. In parallel, education to promote adherence to the current guidelines for cardiac troponin should remain a priority.

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