Clinical and Analytical Standardization Issues Confronting Cardiac Troponin I

Troponin I, a subunit of the ternary troponin complex, is a regulatory protein of the thin filament of striated muscle. Cardiac troponin I (cTnI) is uniquely different from the troponin I found in either slow or fast skeletal muscle. Possessing an additional 31-amino acid sequence at its N terminus, cTnI is found exclusively in the myocardium and, therefore, has been extensively studied as a sensitive and specific serum marker for myocardial injury (1). The forms of cTnI found in the circulation include complexes with troponin T and C (TIC and IC) as well as free subunits (2). In addition, cTnI has been identified in oxidized, reduced, and phosphorylated forms (3, 4). The signals generated by immunoassays developed with anti-cTnI monoclonal antibodies are dependent on the epitope region recognized by these antibodies (5). Thus, protein regions within the cTnI molecule that are susceptible to oxidation, reduction, or phosphorylation or to degradation by the action of proteases are likely to exhibit variable immunoreactivity and even loss of reactivity, leading to altered signal generation in cTnI immunoassays that use antibodies against such regions.

In the December 1998 issue of Clinical Chemistry, Katrukha et al. (6) presented Western blotting and immunoassay data demonstrating the in vivo and in vitro degradation of both the C and N termini of cTnI in human myocardium and serum after acute myocardial infarction (AMI). More importantly, they demonstrated that the signal detected by cTnI immunoassays is highly dependent on the epitope regions recognized by the anti-cTnI antibodies used in the (sandwich) immunoassays. Thus, whether cTnI is complexed with troponin T and or with troponin C as the three-subunit complex TIC or the two-subunit complex IC or as a free subunit, immunoassays designed with antibodies selected against the stable amino acid region between residues 33 and 110 of the cTnI molecule generate a longer lasting signal. Katrukha et al. also presented experimental data that a calcium-dependent cardiac troponin C interaction with cTnI protects the central region of cTnI between residues 33 and 110 from proteolytic degradation, thus allowing preservation of its immunoreactivity.

It has been proposed that the ongoing release of cTnI from the contractile apparatus (which constitutes 95% of cellular cTnI) continues for days during the cellular repair process following AMI. This can explain the prolonged time during which cTnI remains increased in the circulation (7) despite the rapid clearance of cTnI from the circulation. The physiologic clearance of cTnI combined with cTnI assay-dependent loss of immunologic reactivity caused by N- and C-terminal degradation as described by Katrukha et al. (6) provide potentially powerful explanations for intermethod differences in serum and plasma cTnI. Their data (6) raise standardization and clinical issues that affect the clinical use of cTnI as a marker for myocardial injury in laboratory medicine. Standardization of cTnI immunoassays has become an important goal for industry, laboratories, and clinicians. As shown by Wu et al. (3), commercial cTnI assays not only generate different relative responses for a given cTnI concentration, but several assays also generate variable relative response signals dependent on the complexation form of cTnI as well as the oxidation or reduction state of cTnI found in blood. Thus, it is impossible to compare absolute concentrations between manufacturers’ assays without knowing which epitope regions are present in the sample(s) and are being recognized by the assay method. Both the AACC and IFCC have active committees that are addressing the cTnI standardization issue, with the binary complex of IC being the likely first choice of material to explore as a reference material (2, 3). Standardization issues regarding the creatine kinase MB isoenzyme (CK MB) have been addressed previously by an AACC committee, which developed a primary reference material (8). However, the between-manufacturer biases for CK MB were small (<2-fold) compared with the substantial 2- to >20-fold concentration differences between cTnI assays. Because the Dade Stratus II assay was the first FDA-approved cTnI in the United States, most clinical study comparisons by other commercial cTnI assays have been made to the Stratus. Published slopes of regression equations for other manufacturers’ assays (y-axis) vs Stratus are: Beckman Access, slope = 0.100 (9); Behring Opus Plus, slope = 1.59 (9); Abbott AxSYM, slope = 3.50 (9); First Medical, slope = 0.18 (10); Biosite Triage, slope = 0.40 (11); Chiron ACS180, slope = 1.13 (9); and Bayer Immuno 1, slope = 0.81 (9). The wide variation in slopes is partially explained by the findings of Katrukha et al. (6). However, standardization of cTnI assays between manufacturers will not be as simple as determining and optimizing a primary or secondary reference material. On the basis of the observations of Katrukha et al. (6), it appears that the epitopes recognized by antibody pairs used in assays also must be rationalized. Because proteolytic cleavage of cTnI occurs in vivo and in vitro, selection of antibody pairs will influence the interpretation of cTnI concentrations relative to diverse clinical questions, such as the timing of onset of AMI, infarct sizing, reperfusion assessment, and the role of troponins as risk stratification tools.

These analytical challenges raise clinical issues. First, there is a growing database on the use of cTnI for the sensitive and specific detection of AMI, challenging and replacing CK MB in many institutions as the new standard (12). Second, there is an evolving database presenting evidence that cTnI provides a tool for risk assessment in patients presenting with unstable angina (13). One
must be cautious, however, when interpreting the literature based on serum cTnI trends after myocardial injury if comparing different studies that use different cTnI assays. Although this may be understood by those involved in this field, it is not well understood by the majority of clinical chemists, pathologists, and clinicians. Many clinicians (after reviewing a published paper) challenge the laboratory to use the cTnI assay with the lower decision cutoff, without understanding the standardization issues. Furthermore, laboratory administrators may suggest that the clinical laboratory change cTnI assays because of pricing issues, potentially negating whatever database had been established with the then-current assay. The changing reference limits and AMI decision cutoffs that accompany assay switching further confuse clinicians. Switching to recently approved assays that lack the evidence-based literature to support the clinical as well as analytical issues addressed by longer standing assays will soon sour clinicians on the current and future use of cTnI. Although I advocate the use of cardiac troponins to replace CK MB, I recommend caution when replacing CK MB with assays that have not been well studied in clinical trials published in peer-reviewed journals.

I would be remiss if I did not mention cardiac troponin T (cTnT). Several generations of research and commercial cTnT assays have been validated in analytical and clinical trials (1). With the exception of early analytical nonspecificity issues that now have been eliminated in Roche’s second-generation Elecsys cTnT assay (14), cTnT has evolved to be at least equivalent as a marker for AMI (15), and in some studies a more powerful marker for risk stratification in unstable angina patients, compared with cTnI (16). Furthermore, because only one manufacturer provides cTnT assays, intermethod standardization issues have been virtually nonexistent. The evidence-based literature on cTnT can be tracked back 10 years (1), with excellent reproducibility of clinical outcomes involving both testing performed in larger centralized laboratories and the recently released point-of-care testing (POCT) cTnT assay (15). With the development of POCT platforms for cTnT that are capable of whole-blood testing at or near the bedside, laboratories will also need to be cautious about implementing different systems that are not standardized, which could potentially add to clinician confusion when cTnT concentrations are compared between the central laboratory and a bedside (POCT) site. Only Dade Behring (cTnT) and Roche Boehringer Mannheim (cTnT) have platforms available for POCT (Stratus CS and Trop T; respectively) that will correlate with the larger immunoassay analyzers targeted for the central laboratory (Stratus II and RxL from Dade Behring and Elecsys from Roche Boehringer Mannheim, respectively).

As an academic pursuit, the challenge to standardize cTnI assays through antigen and antibody selection will be both scientifically rewarding and potentially confusing to all three sectors: the laboratory, the clinician, and industry. For now, cTnI users should select one assay system; develop a database within their medical center for each myocardial injury-related population studied; and continue to follow comparative trends, not absolute concentration changes, when reading the literature concerning different cTnI assays. For cTnT users, it appears that there is only one assay manufacturer and no standardization issues, studies can focus on clinical interpretation and not on analytical issues. As all troponin users are finding out, the path is not always smooth when attempting to replace a standard such as CK MB. Educating our laboratory peers and our clinical colleagues with convincing scientific evidence will ease the way for the implementation of cardiac troponins as the marker for myocardial injury and risk assessment. The findings of Katrukha et al. (6) are certain to help clarify some of the issues that have confounded cTnI measurements in serum and plasma.

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References

