Standardization of Cardiac Troponin I Assays Will Not Occur in My Lifetime

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I have chosen to address the argument of why cardiac troponin I (cTnI) assays will likely never be standardized. It is not that I do not wish it would happen, but I think the scientific, evidence-based story I will present regarding this cardiac specific protein will speak for itself. As a means to present this argument, I would like to consider the following analogy. I am an avid trail runner. During the second week of June, I set out every morning to run a 10-km rolling trail along the Ammonoosuc River in Bretton Woods, New Hampshire. Although the path I navigated each day was the same, what I observed each morning at 7 AM was dramatically different. It was influenced by the temperature, the wind, rain with thunder and lightning, puddles in rocky crevasses, tree branches covering the path, and, twice, minor course changes due to a fox and 2 black bears that appeared both upstream and downstream on the trail I was navigating. So, what does this analogy have to do with the cTnI story?

cTnl, a protein of 110 amino acid residues with a unique N-terminal sequence that differentiates it from skeletal muscle forms, is part of the myofibril 3 subcomplex (cTnl cTnC cTnT) in the heart with specific structural and regulatory functions (1). Shortly after cell death caused by an acute myocardial infarction (MI), cTnl is released from the damaged myocardium into the extracellular space, and 1–6 h after onset of the index event, cTnl can be detected circulating in the blood (2). Data indicate that cTnl circulates mainly as a binary complex with cTnC in the blood of patients with acute MI, but it also circulates as free cTnl and as a ternary complex, cTnlCT. In addition, C- and N-terminal proteolytic degradation, oxidized and reduced forms, and phosphorylated forms of both free and complexed cTnl have been well described (3).

Immunoassays that use anti-cTnl monoclonal antibodies are dependent on the epitope regions recognized by the antibodies incorporated into each assay. Protein regions within the cTnl molecule that are susceptible to the modifications noted above—as well as the variable nature of epitope-dependent heparin (4), heterophile antibody (5), and autoantibody (6) binding—are likely to exhibit variable immunoreactivity and even loss of reactivity in some cases. Such variability in immunoreactivity can lead to altered signal generation within sandwich-type cTnl immunoassays that use capture and detection antibodies directed against these modified regions. With the acute ongoing release of cTnl during the early post-MI phase, with the ongoing cTnl modifications in both myocardial tissue and blood, and with different clearance mechanisms and rates of clearance for the multiple isoforms of cTnl that accumulate over the first 24 h after an MI, no 2 patients’ blood samples are likely to contain the same cTnl isoform composition (7). In addition, practical issues, such as the preanalytical effects of hemolysis and temperature, have been known to differentially affect assays. Furthermore, as high-sensitivity assays have begun to improve our understanding of the clinical implications of measurable cTnl concentrations in healthy individuals (8), we do not yet have any evidence regarding whether the distributions of cTnl isoforms are the same from person to person, or even whether they remain the same within healthy individuals. For example, some high-sensitivity assays show differences between the sexes, and others do not. Early studies by Wu et al. first showed that commercial assays generated different relative responses for a given cTnl concentration that were based on different responses to the multiple complex forms of cTnl (9). These data showed it was not possible to compare absolute concentrations obtained with assays from different manufacturers.

Standardization of cTnl assays will not be as simple as determining and optimizing a primary or secondary reference material. The goals of the IFCC Working Group on Standardization of Cardiac Troponin I (the opposing point of view in this Point/Counterpoint) appear to be 2-fold (10): first, development of a serum-based secondary reference material...
with cTnI value assignment via a reference measurement procedure; and second, assignment to this material, based on long-term calibration traceability, of a cTnI calibration value through the reference measurement procedure. Although I applaud the efforts of the Working Group (WG) and think these studies will lead to several published reports, the following arguments suggest that the WG will likely fall short of their goals.

For true cTnI immunoassay standardization to be achieved, universal adoption of antibodies, including standardization of both capture and detection/signal antibodies, that are suitable to all manufacturers will be an initial step. That goal is unlikely to be achieved. Furthermore, even when identical antibodies have been used by the same manufacturer on different platforms, there have been conflicting observations that results obtained on these different platforms do not coincide (as seen with cTnI assays marketed by Dade Behring/Siemens and Beckman Coulter). Second, a primary calibration/reference material will need to be produced, along with an accepted mass spectrometry methodology to validate such a material for value assignment and to establish its short- and long-term stability. Although NIST was successful in cooperating with an AACC committee to produce SRM 2921, which is a native cTnI ternary complex produced by HyTest, this material reduced between-assay variation among 13 assays from >40-fold to, at best, 2- to 3-fold and provided suboptimal harmonization (11). The SRM 2921 material allowed some traceability between cTnI assays when used as a calibrator, but use of this material was never made a requirement for assay manufacturers. Recent observations from some members of the WG have demonstrated degradation of the NIST SRM 2921 cTnI material via the use of immunoprecipitation coupled with fluorescence Western blot analysis with cTnI monoclonal antibodies (12).

As these authors stated, “These findings further verified the complexity of cTnI in the pooled patient serum... and the necessity of investigating if the degradation is initially present in the patient sample or occurs during sample process and storage” (12).

The WG has also described a candidate reference procedure based on a multiplexed bead-based immunoassay and SDS-PAGE and claimed equimolar detection of free cTnI, the cTnIC binary complex, phosphorylated and dephosphorylated free cTnI, and the ternary cTnIC complex with or without heparin (13). These findings were predicated on the use of monoclonal antibodies from HyTest (clone 560, which recognizes an epitope at amino acid residues 83–93, and clone 19C7, which recognizes an epitope at residues 41–49) after normalization to SRM 2921. As described above, SRM 2921 is prone to different degrees of degradation—an important fact not addressed by the WG in their quest for standardization. The WG reports a lower limit of linearity of 50 pg/mL, with a lower calibration set point of 100 pg/mL, concentrations that are inadequate for use with high-sensitivity cTnI assays (14, 15), which are capable of measuring concentrations <1 pg/mL and will soon be in clinical practice.

In conclusion, I refer back to my analogy. What nature allowed me to observe day in and day out was never the same during my consecutive morning runs along the same river path. Similarly, owing to nature’s unique mix of cTnI isoforms in each patient sample, what is measured by different cTnI assays in the same patient’s blood will be different, thereby decreasing our current and likely our future confidence in the between-assay comparability of cTnI concentrations.

As an aging athlete, the differences in my daily running experiences do not bother me. I am just happy that I am still able to run every morning. Laboratorians and clinicians, likewise, should not get bogged down with cTnI standardization. Rather, they should develop a clear understanding of the clinical and analytical evidence for their chosen assay and be happy that the technological improvements that have led to the precise detection of low cTnI concentrations also will lead to better patient care.

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References