Isoenzymes of creatine kinase (CK) have been used for the diagnosis of patients with acute myocardial infarction for more than 30 years. While CK-MB continues to be widely used, cardiac troponins T (cTnT) and I (cTnI) are rapidly becoming the new “gold standard” markers for use in patients with acute coronary syndromes. The success of troponin is due to the fact that assays have high clinical sensitivity and specificity for detection of myocardial injury. The sensitivity is improved over existing markers such as CK-MB because the myocardial tissue content of cTnT and cTnI is considerably higher (10.8 and 4-6 mg/g wet weight, for troponins, respectively, vs 1.4 mg/g for CK-MB). Thus, for an increment of cardiac damage, more troponin is released. The specificity is better because skeletal muscle troponin isoforms have enough differences in the primary amino acid sequences that immunoassays using monoclonal antibodies toward the cardiac forms do not cross-react with the corresponding skeletal muscle forms. This is unlike CK-MB and myoglobin for which the skeletal muscle isoforms are identical to their cardiac counterparts. Thus, normal blood contains very little cardiac troponin, and low cutoff concentrations can be used. This further enhances the sensitivity of troponin assays. Thus, the combined measurement of CK-MB and cTnT/cTnI provides the clinician with excellent and reliable markers of myocardial injury.

The study conducted by Messner and colleagues in this issue of the Journal raises new questions about the absolute specificity of cardiac troponin assays. By using muscle biopsy specimens of 24 patients with skeletal muscle myopathies (biopsy confirmed), they demonstrated that in addition to messenger RNA (mRNA) for skeletal muscle troponin, mRNA for cTnT and cTnI was present in 17% and 14% of their samples, respectively. However, while provocative, this study has 2 weaknesses: failure to detect the cardiac form protein by Western blot analysis and detection of increased levels in the plasma of these patients. Nevertheless, these observations prompt a more fundamental question: What do we really know about the release of cardiac troponin and its appearance in blood? Although more than a dozen qualitative (point-of-care) and quantitative commercial assays for cTnT and cTnI are approved by the US Food and Drug Administration, and these tests have been used in routine clinical practice for 5 to 7 years, much is still to be learned about the basic analytic chemistry and biochemistry of these proteins.

Issues of specificity have been raised since release of the very first cTnT assay, specifically in regard to patients with acute renal failure, chronic renal failure, and skeletal muscle disease. These studies showed that the incidence of positive cTnT results at about 50% was substantially higher than for cTnI in blood collected from the same patients (<10%). Soon thereafter, it was shown that many of these positive cTnT results were caused by nonspecific binding of skeletal muscle troponin T. The development of second-generation assays has reduced the incidence of positive results in chronic renal failure to less than 20%. By using Western blot analysis, some have suggested that regenerating skeletal muscle and renal tissue can reexpress cTnT, an observation that does not seem to occur for cTnI. However, when the specific monoclonal antibodies used in the current generation Roche (Nutley, NJ) cTnT assays were studied, no false-positive results were produced in these tissues. The best indicator of whether increased cTnT in renal failure is due to a lack of specificity or represents true myocardial injury that may be missed by cTnI may be to compare the mortality rate, eg, at 1 year, of renal failure patients with abnormal troponin concentrations. In this regard, risk stratification studies to date have been controversial: some studies indicate poor risk with a positive troponin result, while others indicate no prognostic use.

Nonspecificity for troponin assays also can be the result of analytic interference. The presence of fibrin can retard the separation of bound from free analytes, producing falsely positive results. Because the laboratory is under increasing pressure to improve turnaround times for reporting results of cardiac markers, it is not uncommon to see bench technologists loading samples on instruments for testing before the samples are ready. This problem can be eliminated by...
allowing enough time for full clot retraction and vigorous centrifugation of the sample before analysis.\textsuperscript{14} The presence of heterophile antibodies, such as is caused by rheumatoid factor, also can produce false-positive results.\textsuperscript{15,16} These antibodies bind the captured and labeled antibodies used in the troponin assays, thereby generating a positive analytic signal in the absence of the analyte. Next-generation troponin T and I assays have reduced these problems with the incorporation of the appropriate blocking substances within the reagent formulation.\textsuperscript{17}

The data from Messner et al\textsuperscript{3} also raise new fundamental biochemical questions about the distribution of troponin in diseased tissues and its release after myocardial injury. Early work suggested that troponin first is released as a ternary complex of cTnT-I-C, which subsequently degrades into a binary complex of cTnI-C and free cTnT (with little or no free cTnI).\textsuperscript{18} However, more recent data suggest that during cardiac ischemia, troponin may be degraded into small fragments within the myocyte, which might be released into blood during reversible ischemia.\textsuperscript{19} These observations go against the prevailing view that myocardial proteins are released only after irreversible injury.\textsuperscript{20} Data in animal models have shown that CK is not released in reversible injury.\textsuperscript{21} Corresponding data for cardiac troponin are only now being generated. Preliminary data on cardiac troponin suggest that release might be possible in reversible injury.\textsuperscript{22,23} One hypothesis is that under ischemic conditions, troponin is degraded in situ into lower molecular weight subunits, and these epitopes are maintained such that troponin assays can recognize these fragments.\textsuperscript{24}

It is clear that more research is necessary to characterize this very complex pair of proteins. Although Messner et al\textsuperscript{3} have raised some interesting questions, can we necessarily infer that the presence of cardiac troponin mRNA present in regenerating skeletal muscle tissue necessarily means that serum assays for cardiac troponin will be inherently nonspecific? In their own studies, they were unable to demonstrate positive troponin results in any of the serum samples of patients with skeletal muscle myopathies; however, they did not report on the majority of patients with Duchenne muscular dystrophy who clearly demonstrate mRNA. If everything can be explained at the genomic level, then the entire emerging biochemistry field of proteomics may be superfluous.\textsuperscript{25} The fact that there are many changes that can occur from gene transcription and translation to production of the final functional protein suggests that it is premature to draw conclusions of specificity for troponin assays in the majority of patients, but caution is needed when making conclusions about patients with severe myopathy, based on the interesting but very preliminary observations made by Messner et al.\textsuperscript{3}

References


